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KINETICS OF BETA-CAROTENE DEGRADATION
IN AN OXYGEN ENVIRONMENT

By

Maria Beatriz Abreu Gloria

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

KINETICS OF BETA-CAROTENE DEGRADATION IN
AN OXYGEN ENVIRONMENT

By

Maria Beatriz Abreu Gloria

Beta-carotene is very susceptible to oxidation due to its high degree of unsaturation. Degradation of beta-carotene in dehydrated foods affects their attractive color, their nutritive value and their flavor leading to unacceptability and shorter storage life. The purpose of this research was to develop kinetic models for beta-carotene loss and degradation product formation in dehydrated foods. Analytical methods were developed for separating alpha- and beta-carotene, recovering beta-carotene from degraded model and food systems and separating and identifying volatile degradation products. Kinetic models were developed for beta-carotene loss and product formation in a model system and in dehydrated carrots.

The determination of beta-carotene by simple measurement of absorbance at 450nm (the commonly used method) was proven to be invalid for the monitoring of beta-carotene loss as oxidation products also absorb at that same wavelength. A normal phase HPLC procedure, using a gradient

of acetone in hexane, was adapted to separate beta-carotene from its degradation products. A second HPLC method was improved to separate alpha- and beta-carotene. Volatile degradation products were collected by a Tenax-GC trap system and identified by GC/MS analysis. Oxidation products comprised mainly of C₉, C₁₀, C₁₁ and C₁₃ compounds, originated from cleavage of bonds 6-7, 7-8, 8-9 and 9-10 in beta-carotene. The major degradation components were alpha-isophorone, beta-cyclocitral, beta-ionone, 5,6-epoxy beta-ionone and dihydroactinidiolide.

Three approaches were used for kinetic modeling: a first order reaction mechanism, a free radical recombination mechanism involving oxygen attack in several steps, and a 1.5 order reaction. The first order model included product formation rates and was the most satisfactory for the loss of beta-carotene over the temperature range investigated. The production of dihydroactinidiolide (one of the major oxidation products of beta-carotene) correlated well with beta-carotene concentration up to 50% loss of beta-carotene. Therefore, measurement of dihydroactinidiolide was considered an alternate method for monitoring beta-carotene loss.

The kinetic model approach was applied to the loss of alpha- and beta-carotene and production of volatile oxidation products in dehydrated carrots. The loss of beta-carotene in dehydrated carrots was much lower than that observed for model systems. The loss of alpha-carotene was

lower than the loss of beta-carotene. Volatile products identified in model system studies represented 40 to 50% of the total volatile fraction in dehydrated carrots. The model gave good correlation between beta-carotene loss and production of dihydroactinidiolide as a function of temperature.

**To my husband, Ubiratan
and
to my daughters,
Maria and Janaina.**

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LIST OF SYMBOLS

$h\nu$	- quantum energy
Sens	- photosensitizer
Δ	- heat
β	- beta
k	- rate constant
E_a	- activation energy
% RH	- percent relative humidity
?	- unknown value
RH	- beta-carotene
R.	- beta-carotene free radical
ROO.	- peroxy free radical
k_i	- initiation rate constant
K	- oxygen step equilibrium constant
k_p & k'_p	- propagation rate constants
k_t , k'_t , k''_t	- termination rate constants
t	- time
P_i	- intermediate products
D_f	- final products
b_o	- reactivity of beta-carotene
b_i	- reactivity of intermediate products
c	- $a(b-b_o)^{1/2}$
a	- $k_p/k_t^{1/2}$
E	- $1 - [(RH)/(RH_o)]^{1/2}$
T	- $a(b-b_o)^{1/2}$
BET	- Brunauer-Emmet-Teller equation
C	- beta-carotene concentration
C_o	- beta-carotene initial concentration
k_c	- rate of beta-carotene degradation
k_v	- rate constant for volatile formation
r_v	- rate of volatile formation
P_v	- volatile concentration
C_1	- constant
$\bar{\sigma}$	- effective rate constant
r^2	- correlation for linear regression

INTRODUCTION

Due to its high degree of unsaturation, beta-carotene is very susceptible to oxidation. Degradation of beta-carotene in dehydrated foods affects the attractive color, the nutritive value and flavor of foods, leading to unacceptability and shorter storage life. Accurate monitoring of beta-carotene in food systems would lead to (i) improved information on the effects of storage on beta-carotene loss, (ii) accurate prediction of vitamin content, giving the processor assurance of meeting label claims, and (iii) prediction of off-flavor development and acceptability of the product.

There is no consensus on mechanisms for oxidative degradation of beta-carotene. Therefore, the purpose of this research was to develop kinetic models for beta-carotene loss and degradation product formation in dehydrated foods.

The specific objectives of this investigations were:

1. To develop methodology for the measurement of beta-carotene loss in model and in food systems.
2. To identify volatile degradation products that originate from the oxidation of beta-carotene.

3. To evaluate the kinetics of beta-carotene loss in model and food systems.

4. To correlate beta-carotene loss with production of volatile oxidation products in model and food systems.

REVIEW OF LITERATURE

1. CAROTENOIDS

Carotenoids are a group of lipid soluble compounds responsible for many of the yellow and red colors of plant and animal products. They are very widespread and occur naturally in large quantities (Klaui, 1981).

Carotenoids have received a great deal of attention from early researchers because beta-carotene is a precursor of vitamin A. Beta-carotene yields two molecules of vitamin A by cleavage at the center of the molecule. Alpha-carotene functions as a precursor for one molecule of vitamin A. Besides their normal vitamin A function, recent research has shown that some pro-vitamin A compounds may have anticancer, antiaging and antiulcer properties (Peto et al., 1981) and have been used as protective agents in certain skin diseases aggravated by light (Mathews-Roth, 1981).

Carotenoids contained in natural extracts (from carrots, palm oil and butter fat) have been employed as food colorants. These colorants have the added benefit of providing vitamin A activity. These natural extracts may be supplemented with synthetic carotenoids in a number of

foods. Synthesized beta-carotene is currently approved by the FDA for addition to human foods. There is no legal limit on the addition of beta-carotene to foods (Francis, 1985).

Due to their high degree of unsaturation, carotenoids are very susceptible to oxidation. The severity of oxidation depends on the environmental conditions to which the food is exposed. In intact living tissue, the stability of the pigment is a function of cell permeability and the presence of protective components. Enzyme systems which degrade carotenoids rapidly are present in many tissues.

Lipoxygenases and peroxidases are responsible for the oxidation and degradation of carotenoids in a number of foods. Carotenoid losses during processing and storage of foods are of commercial significance. Carotenoid degradation is desirable in the production of tea and tobacco as its oxidative degradation products play an important role in their flavor and aroma (Wahlberg et al., 1977 and Sanderson et al., 1971). However, degradation of carotenoids affects the attractive color of foods, their nutritive value and flavor, leading to unacceptability and shorter storage life of foods. Dehydrated foods are the most susceptible to loss of carotenoids because of their tendency to undergo oxidation (Labuza et al., 1970).

The mechanism of carotenoid oxidation in processed foods is complex and depends on many factors. The pigments may autoxidize by reaction with atmospheric oxygen at rates dependent on light, heat, and presence of compounds

possessing pro- and antioxidant character. The reaction is believed to be caused by free radical formation with three distinct steps involved: initiation, propagation and termination. Carotenoids can act as antioxidants or prooxidants depending on the environment (McConnell and Esselen, 1947 and Du and Armstrong, 1970).

The control and monitoring of carotenoid oxidation is important to the dehydrated vegetable processor, as there is a sizeable market for these products. The development of a carotenoid stability kinetic model would (i) provide an accurate prediction of vitamin content for dried foods during storage, giving the processor the assurance of meeting label claims, and (ii) allow prediction of off-flavor development and acceptability of the product.

2. ANALYTICAL METHODS

Analysis of Carotenoids.

The quantitative determination of carotenoids is very difficult. Carotenoids are subject to isomerization and decomposition requiring special care to minimize their exposure to air, heat, light or reacting chemicals during analysis (Thompson, 1986). The simplest form of carotenoid analysis consists of estimating pigment content by direct spectral measurement. Such measurements are subject to light scattering, nonuniform distribution of the pigment, and

interference from other pigments (De Ritter and Purcell, 1981). Other methodologies available for determining carotenoids can be grouped into three categories - open column, thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC).

The open column techniques (Purcell and Water, 1968; Rodriguez et al., 1976; Gebhardt et al., 1977 and AOAC, 1980) of which the AOAC procedure is the most widely used, are time consuming, are not as quantitative as other methods due to numerous steps, and for most part cannot distinguish between alpha- and beta-carotene. They tend to overestimate pro-vitamin A activity where a complex mixture exists (Simpson et al., 1985). The thin layer procedures are lengthy and the separation and quantitation of the carotenes is difficult (Davies, 1965 and Baloch et al., 1977a). They also lack reproducibility, sensitivity and accurate quantitation (Zakaria and Simpson, 1979). TLC is not suitable for quantitative analysis because carotenes are destroyed by exposure to air (Thompson, 1986).

High pressure liquid chromatography techniques are generally considered to be the quickest, simplest and the most reproducible method of analysing carotenoids (Sweeney and Marsh, 1971; Fiksdahl et al., 1978; Zakaria and Simpson, 1979; Stancher and Zonta, 1982; Bushway and Wilson, 1982 and Hsieh and Karel, 1983). The literature is overwhelmed with methods, however many modifications and adaptations are required to reproduce them. The most popular packing has

been C₁₈. The most extensive system has been developed by Sweeney and Marsh (1971). However, it is lengthy as it involves an initial open column step followed by two HPLC systems not commercially available.

Analysis of Volatile Degradation Products.

Flavor research has made significant progress since the late 1960's due to the development and application of techniques and instrumentation particularly well suited to the separation and identification of trace amounts of volatile compounds (Flath et al., 1981). The isolation of volatile compounds from the food matrix is critical to their identification as it will have profound effects on the resulting profile. Several isolation techniques have been developed in order to obtain a volatile mixture that represents the characteristic flavor of the food.

Distillation, extraction, adsorption and other procedures are commonly used. According to Sugisawa (1981), isolation and concentration by adsorption and by simultaneous distillation extraction have been the major means of flavor isolation and concentration.

During distillation, steam is introduced into the sample, increasing the volatility of the flavor components. This method is effective as it enables the separation of volatile components from macromolecules and non-volatile components of the food system. However, according to Flath et al. (1981), there is a great potential for artifact

formation during the heating process (the volatile components of the isolate will be characteristic of the cooked rather than the raw product). Another disadvantage is that water will be present in the distillate, requiring further solvent extraction. The solvent used in the extraction of volatiles (hexane, methyl butane, diethyl ether, dichloromethane) must be of high purity and be inert so it will not react with the flavor compounds, changing the flavor profile or interfering with the chromatogram as concentration is required (Heath and Reineccius, 1986). Vacuum distillation can be used to minimize artifact formation arising through thermal degradation. Likens and Nickerson (1964), developed a simultaneous distillation extraction process for the analysis of hop oil constituents. There was an increase in efficiency of flavor isolation (the extraction step was eliminated). However there was still potential for artifact formation through thermal degradation.

Headspace analysis can be used to detect volatiles in the gas phase of a sealed vial containing the foodstuff. Direct headspace sampling lacks sensitivity as large headspace volumes must be used, resulting in poor chromatography and allowing only the detection of major components (Reineccius, 1985). Porous polymer traps such as Porapak and Tenax GC have been used to trap the volatiles from the headspace (Simon et al., 1980; Heydanek and McGorinn, 1981a,b; Galt and MacLeod, 1984; Min and Kim, 1985

and Olafsdottir et al., 1985). Adsorbents should be chosen on the basis of their selective adsorption characteristics. Tenax GC is hydrophobic in nature and has good thermal stability. It has low affinity for low molecular weight alcohols. It can be employed in the collection and desorption of volatiles of higher molecular weight compounds (Olafsdottir et al, 1985). Volatiles collected in the porous polymer traps can be stored for two weeks at 0 to 4°C (Jennings and Filsoof, 1977).

Appropriate precautions concerning the preconditioning of the trap, mode of elution of the adsorbed volatiles and artifactual formation of flavor compounds must be considered. Temperatures employed for conditioning, adsorption and regeneration should not induce the development of artifacts which can be incorrectly identified as constituents of the product. Lewis and Williams (1980) observed 59 compounds when they held Tenax GC above their normal operating temperature. These compounds were eluted from the polymer traps after regeneration of the traps and prior to their use for the collection of volatiles. In comparison to the continuous solvent extraction method described by Likens and Nickerson (1964), entrapment of volatiles is less time consuming, less costly and less susceptible to thermal artifact formation.

3. MECHANISMS OF BETA-CAROTENE DEGRADATION

There is no consensus about the mechanism of the formation of degradation products from polyenes in the presence of oxygen, in spite of the fact that highly unsaturated compounds exposed to air are very unstable (Ohloff, 1972). Some possible pathways for the oxidation of beta-carotene have been proposed:

Oxidation with Metal Oxides.

According to Wilberg and Saegerbarth (1957), oxidation with metal oxides (KMnO_4) involves the transitory formation of a five membered intermediate. The formation of a space-requiring transition state would favor those sites of least steric hindrance. These might give the most stable transition state, even though these sites may not be the centers of the highest electron density.

Oxygen Attack on the 5,6-Double Bond.

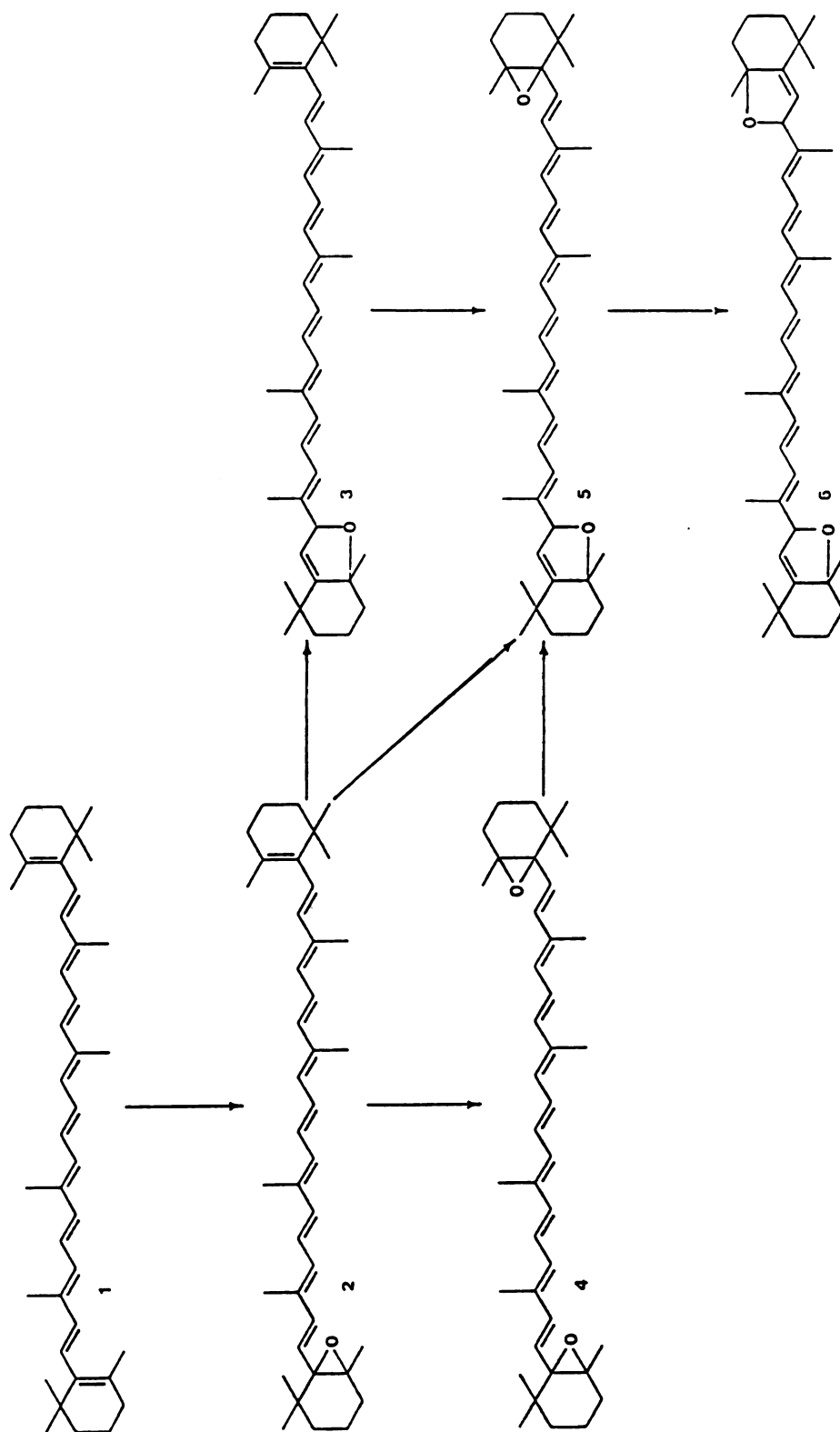
El-Tinay and Chichester (1970) investigated the initial site of the oxygen attack on the beta-carotene molecule during molecular oxidation (in the dark at 60°C). The predominant formation of epoxides suggested that the initial site of oxygen attack occurred at the terminal double bonds. Marty and Berset (1986) also identified epoxides during thermal degradation of beta-carotene (180°C in sealed glass tubes and extrusion cooking) and proposed the reaction

sequences outlined in Figure 1 for their formation. The 5,6 position would be the site of oxygen attack causing epoxidation. This is in agreement with the predictions of the oxidation of a conjugated system, since in a conjugated system the highest electron density is found in the terminal double bonds, with a progressive depletion of electron density as the central double bond is approached (Hunter and Krakenberger, 1946; El-Tinay and Chichester, 1970 and Marty and Berset, 1986). Subsequently, a conversion of the epoxide function to a furanoid oxide can occur, the final product being beta-carotene 5,8,5',8'-diepoxide. According to El-Tinay and Chichester (1970), the reaction between oxygen and beta-carotene may go through an activated dipole association complex formed in an equilibrium step. This activated complex may then decompose into products or go back to free oxygen and beta-carotene.

Oxidation with Singlet Oxygen.

Assuming that biological oxidations proceed along a similar pathway to that of oxidation reactions with singlet oxygen, Ohloff (1972) proposed that singlet oxygen could react with beta-carotene either by manner of (A) an ene reaction, (B) by direct cycloaddition to the double bond - dioxetane mechanism, or (C) through the formation of a perepoxide (Figure 2). All peroxides are unstable and would generate the same fragments. Monocyclic isoprenoid flavor

Figure 1. Reaction sequence for the formation of beta-carotene epoxides during heating. 1- Beta-carotene; 2- beta-carotene-5,6-epoxide; 3- beta-carotene-5,8-epoxide; 4- beta-carotene-5,6,5',6'-diepoxide; 5- beta-carotene-5,8,5',6'-diepoxide; 6- beta-carotene-5,8,5',8'-diepoxide (Marty and Berset, 1986).



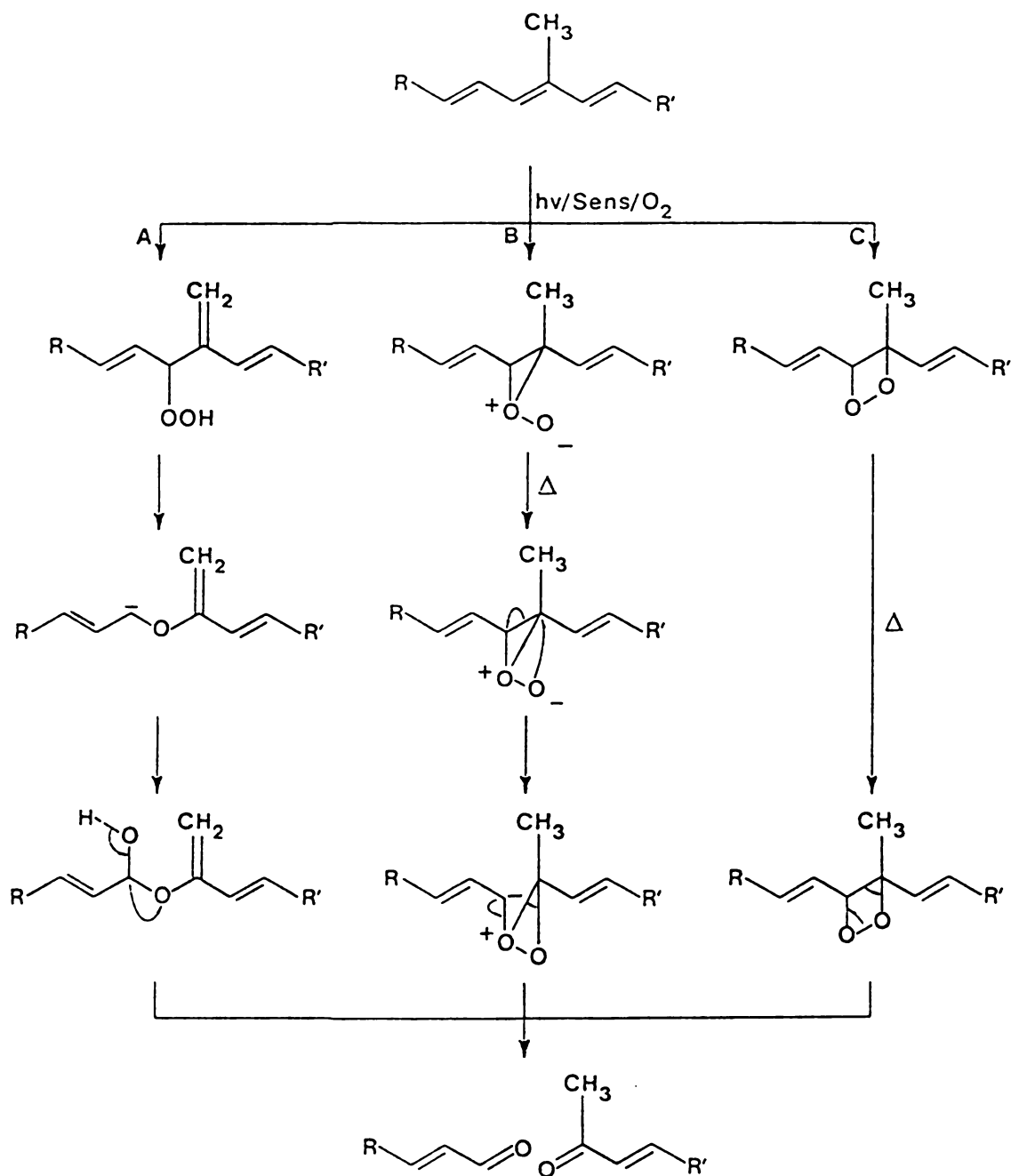


Figure 2. Mechanism of photosensitized oxidation of polyenes with fragmentation of intermediates to dicarbonyl compounds. A- Ene mechanism; B- Peroxy-epoxy mechanism; C- Dioxetane mechanism (Ohloff, 1972).

compounds represented in Figure 3, could be formed by fragmentation of the oxidation products resulting from the action of singlet oxygen on beta-carotene.

4

Oxidation with Triplet Oxygen.

Beta-ionone has been identified as a thermal degradation product of beta-carotene (Isoe et al., 1969; Ohloff, 1972 and Ouyang et al., 1980). Ouyang et al. (1980) isolated apo-carotenals and apo-carotenones from the simulated deodorization of palm oil and suggested that Ohloff's dioxetane mechanism (Figure 4) could be used to explain the formation of such oxidation products. When the molecule is attacked by oxygen at positions (13)C=C(14), (15)C=C(15') and (14')C=C(13'), beta-13-apo-carotenone, beta-15-apo-carotenal and beta-14-apo-carotenal are formed.

Mechanism of Thermal Degradation.

The mechanism for the formation of toluene, m- and p-xylene, and 2,6-dimethylnaphthalene has been proposed by Edmunds and Johnstone (1965) and advanced by Schwieter et al. (1969). It is said to involve simple cyclization and elimination with the formation of a four membered ring intermediate. Mechanistic investigations led Ohloff (1972) to conclude that the fragmentation of beta-carotene must be considered as the result of a series of successive thermal reactions as shown in Figure 5. The reaction is initiated by a sigmatropic 1,5-hydrogen shift of the allylic methyl

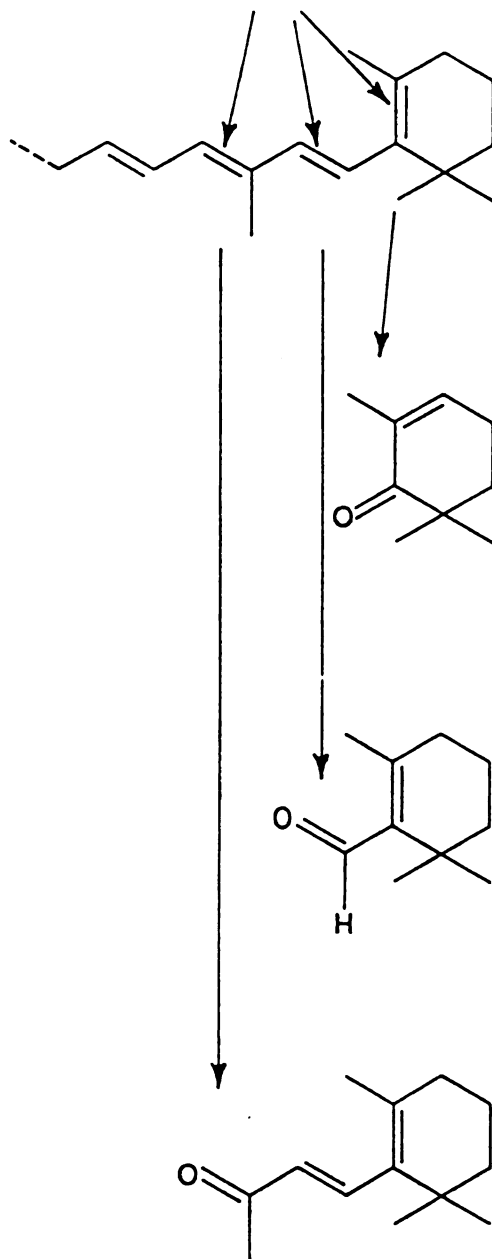


Figure 3. Possible formation of oxygen-containing fragments in a carotenoid model (Ohloff, 1972).

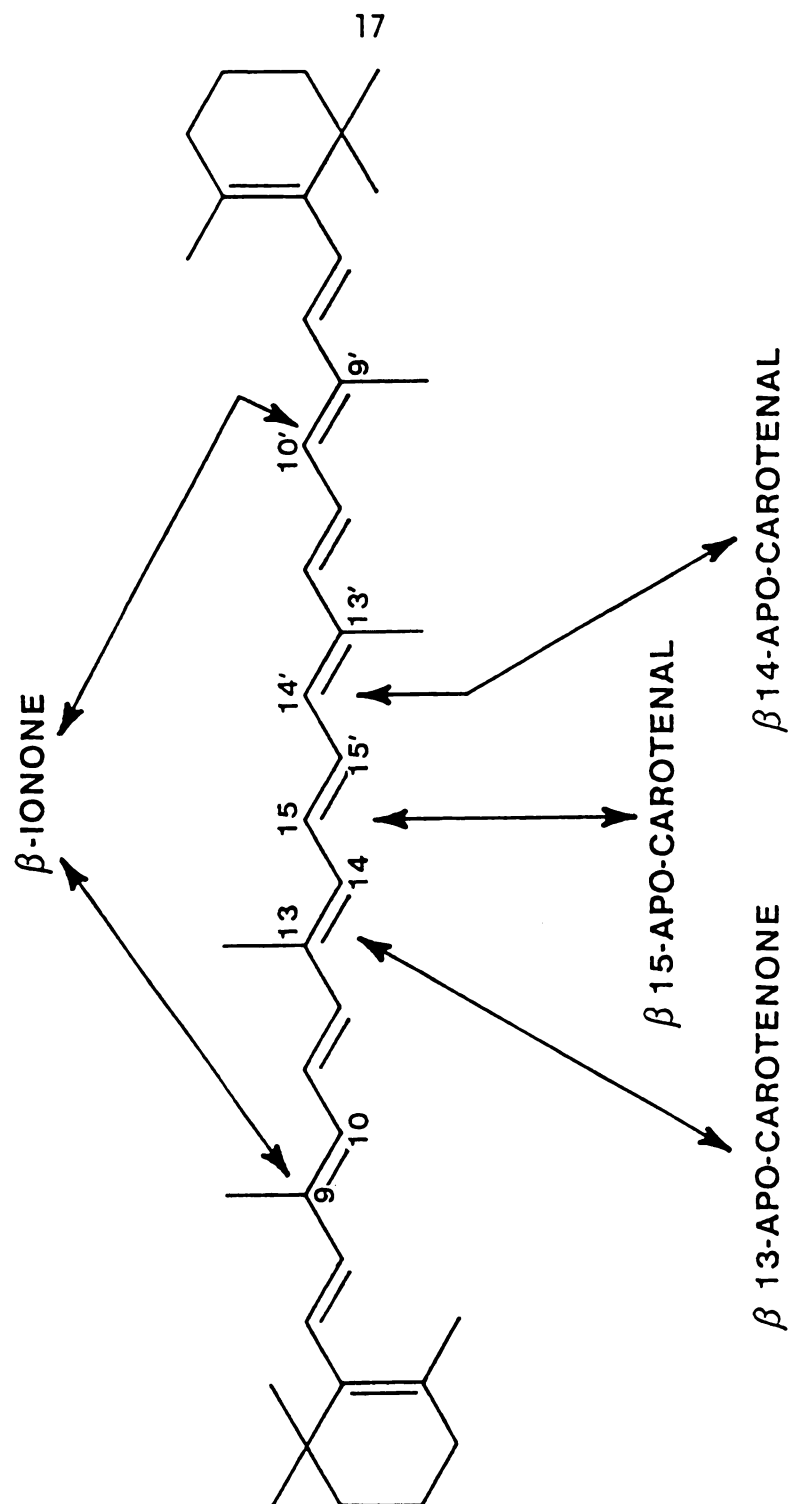


Figure 4. Relationship between the attacking positions by oxygen and the formation of three carbonyl compounds (Ouyang et al., 1980).

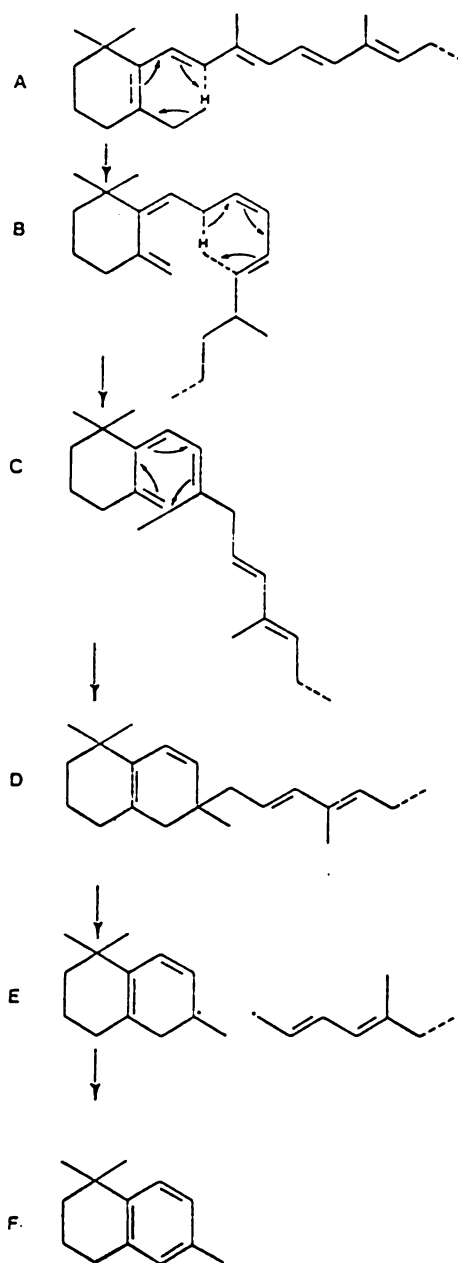


Figure 5. Mechanism of the thermal ionene formation from beta-carotene by a sequence of sigmatropic hydrogen shifts (A $\rightarrow$ B $\rightarrow$ C), an electrocyclic rearrangement (C $\rightarrow$ D), and a homolytic fission reaction (E $\rightarrow$ F) (Ohloff, 1972).

hydrogen of the cyclic part in beta-carotene to the first double bond of the side chain. After isomerisation of the first tri-substituted double bond in the chain from the trans- to the cis-position, the second sigmatropic 1,5-hydrogen shift can then take place. The resulting intermediate (C) is converted very rapidly into the bicyclic compound (D) as a result of an intramolecular Diels-Alder reaction. The aromatization is due to the very weak gamma-bond between the two double bond systems. This bond is homolytically cleaved, as shown in E. A subsequent intermolecular hydrogen transfer finally results in the formation of ionene. According to Ohloff (1972), the formation of toluene, m-xylene, and 2,6-dimethylnaphthalene can be explained in a similar manner, as result from the fragment radical of the side-chain E.

4. KINETICS OF BETA-CAROTENE DEGRADATION

In a number of studies it was mentioned that the oxidation of beta-carotene represents an autocatalytic process (Alekseev et al, 1968; Finkel'stein et al., 1973 and Stefanovich and Karel, 1982). The accelerating action of certain substances, for example, metals or compounds containing metals, as well as free radical initiators, together with the possibility of inhibiting the oxidation of beta-carotene by addition of compounds that are inhibitors

of free radical reactions, are evidence of the free radical chain character of the process of oxidation of beta-carotene (Alekseev et al., 1968).

In the analysis of the kinetics of beta-carotene degradation, two approaches have been used: (1) a simplified first order reaction approach (Chou and Breene, 1972; Arya et al., 1979b; Ramakrishnan and Francis, 1979a and Stefanovich and Karel, 1982) and (2) a free radical recombination mechanism involving oxygen attack in several steps (Alekseev et al., 1968; Labuza, 1971; Finkel'stein et al., 1973, 1974; Gagarina et al., 1970; Stefanovich and Karel, 1982; Goldman et al., 1983 and Saguy et al., 1985).

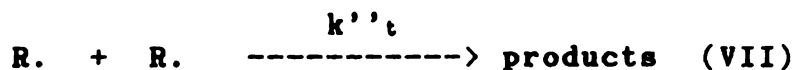
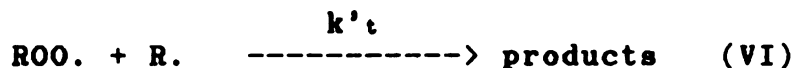
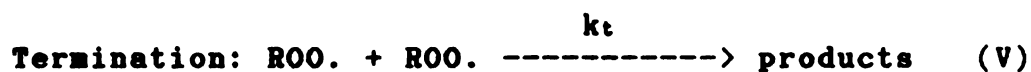
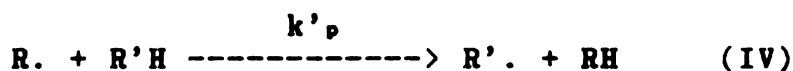
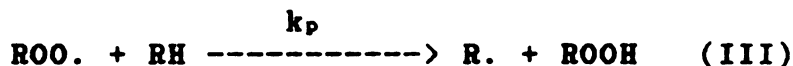
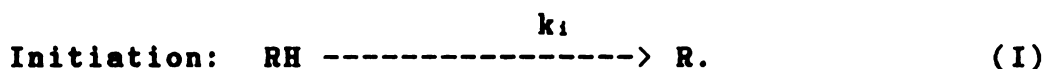
In the first case, Chou and Breene (1972), Baloch et al. (1977c), Ramakrishnan and Francis (1979a) and Arya et al. (1979a) considered that after an induction period which decreases with increasing temperature, the rate of decoloration follows first order kinetics. First order kinetics is expected if beta-carotene and oxygen are the only reactants and if a large excess of oxygen is present. First order rate constants for beta-carotene degradation in model systems are summarized in Table 1. There is much variability among measured rate constants. Beta-carotene concentration was determined by simple measurement of absorbance at 450 nm. The rates of reaction as well as activation energy observed by Ramakrishnan and Francis (1979a) and Stefanovich and Karel (1982) are significantly higher than those observed by others.

Table 1. First order rate constants^a and activation energies for beta-carotene degradation in model systems.

References	Experimental Conditions		Rate Constant	Activation Energy E _a $\frac{\text{kcal}}{\text{mol}}$
		T°C	k (h ⁻¹)	
El-Tinay and Chichester, 1970	.1 mg/ml beta-carotene	60	3.93 x 10 ⁻²	10.2
		70	6.12 x 10 ⁻²	
	toluene	80	10.54 x 10 ⁻²	
		90	14.03 x 10 ⁻²	
Chou and Breene, 1972	0.5% beta-carotene	5	1.2 x 10 ⁻³	9.7
	dry	20	4.4 x 10 ⁻³	
	Avicel	35	8.8 x 10 ⁻³	
Baloch et al., 1977c	? beta-carotene	37	17 x 10 ⁻³	10.3
	31% RH	44	23.9 x 10 ⁻³	
	microc. cellulose	50	36.6 x 10 ⁻³	
Ramakrishnan and Francis, 1979a	? beta-carotene			
	dry	25	3.96 x 10 ¹	
	Avicel			
Arya et al., 1979a	1.7 mg/g beta-carotene			
	33% RH	16-32	(3.4 x 10 ⁻³)*	
	microc. cellulose			
Stefanovich and Karel, 1982	.14/.57/.89 mg/g beta-carotene	60	0.22/0.23/0.21	23.9/28.9/25.6
	dry	70	0.71/0.85/0.83	
	Avicel	80	1.43/1.73/1.54	

* Values in parentheses are calculated first order values from literature data.

In the second case, the oxidation of beta-carotene in thin films was considered to be an unbranched chain process with a nonstationary or acceleration period associated with the increase in concentration of free radicals, and a period in which the oxidation of beta-carotene takes place at a constant rate (Finkel'stein et al., 1973). During the oxidation of carotene, the intermediate products formed are also consumed by chain reactions (Gagarina et al., 1970). It is assumed that all these intermediate products have the same reactivity. The degradation of beta-carotene is then given:



where: RH - beta-carotene (unreacted)

R. - beta- carotene free radical

ROO. - peroxy free radical

k_i - initiation rate constant

K - oxygen step equilibrium constant

k_p & k'_p - propagation rate constants

k_t , k'_t & k''_t - termination rate constants

Initiation (Reaction I)

The initiation by means of singlet oxygen has been proposed by Ohloff (1972). In the case of autoxidation, the initiation step is obscure. Trace metals, if present could be responsible for primary initiation. Increased temperature can be responsible for direct attack of oxygen (Shelton and Vincent, 1963). Ultraviolet light would be a good initiator if a proper sensitizer was present. It is not known if the initial formation of radical is monomolecular (Stefanovich and Karel, 1982 and Goldman et al., 1983) or bimolecular (Alekseev et al., 1968).

Propagation (Reactions II-IV)

In the propagation step, the free radicals generated will react with oxygen to give alkyl peroxy radical (II). According to El-Tinay and Chichester (1970) and Ohloff (1972), epoxidation could proceed through addition of oxygen to the double bond of the carbons 5-6, as it is the site of higher electron density. In the presence of singlet oxygen, the dioxetane mechanism, the peroxy-epoxy mechanism or even the ene mechanism could proceed (Ohloff, 1972). The

dioxetane mechanism has also been suggested to be responsible for beta-carotene degradation during deodorization (Ouyang et al., 1980). However no one has investigated the peroxides formed and there is no evidence of their formation.

Termination (Reactions V-VII)

Termination leads to the formation of non radical products. It is considered to be a second order termination (Saguy et al., 1985).

Considering the degradation of beta-carotene in excess oxygen, reactions (I), (II), (III) and (V) are applicable. Assuming a stationary state method, it follows that except for the initial and final stages of the reaction, the rate of change of the intermediate is equal to zero. The rate of consumption of beta-carotene, at high oxygen pressure, is not dependent on oxygen pressure (Alekseev et al., 1968):

$$\frac{dRH}{dt} = r_i + k_p \frac{r_i^{1/2} (RH)}{k_t^{1/2}} \quad (1)$$

but $r_i = k_i (RH)$

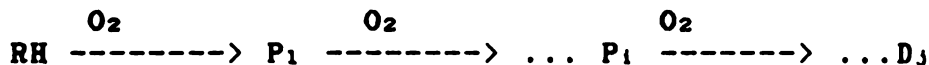
$$\text{then, } \frac{dRH}{dt} = (RH) \left(k_i + \frac{k_p}{k_t^{1/2}} k_i^{1/2} (RH)^{1/2} \right) \quad (2)$$

where r_i = initiation rate

t = time

Several investigators have observed that the maximum rate of absorption of oxygen exceeds the maximum rate of beta-carotene degradation, and that the absorption of oxygen continues for a long time after the disappearance of the

initial carotene (Gagarina et al., 1970 and Teixeira Neto et al., 1981). This means that the consumption of oxygen is connected with the oxidation of both carotene and intermediate products:



In such a process, the rate of formation of free radicals (initiation rate), r_i , would be the sum of the initiation rate of radicals formed by the unreacted carotene and by the intermediate products (Gagarina et al., 1970):

$$r_i = b_0(\text{RH}) + \sum_{i=1}^k b_i(\text{P}_i) = b_0(\text{RH}) + b[(\text{RH})_0 - (\text{RH})] \quad (3)$$

where b_0 and b_i are the kinetic parameters determining the reactivity of the hydrocarbon and of the products in reactions leading to the formation of free radicals. Gagarina et al. (1970) assumed that the reactivity of all the points of oxygen attack were identical, i.e., $b_1 = b_2 = \dots = b_i = b$. Long chain reactions of oxygen along the conjugated double bond chain would result in highly oxygenated degradation products. Taking into account expression (3) for the change in r_i during the oxidation, the equation for the rate of consumption of carotene has the form

$$-\frac{d\text{RH}}{dt} = b_0\text{RH}_0 + b(\text{RH}_0 - \text{RH}) + \frac{k_p}{k_t^{1/2}}(\text{RH}) [b_0\text{RH}_0 + b(\text{RH}_0 - \text{RH})]^{1/2} \quad (4)$$

or

$$dE/dT = (1 - E) [E_0 + E_1]^{1/2}$$

where $E = 1 - [(RH)/(RH_0)]^{1/2}$ and $T = a (b - b_0)^{1/2} t$ are dimensionless variables, and $E_0 = b_0/(b - b_0)$. Equation (4) determines the ratio between the maximum rate and the initial concentration of hydrocarbon in a manner that agreed with the experimental relationships. Gagarina et al. (1970) used the following equation to describe the change in beta-carotene concentration with time:

$$\ln [1 + (1 - RH/RH_0)^{1/2}] / \ln [1 - (1 - RH/RH_0)^{1/2}] = c RH_0^{1/2} t \quad (5)$$

where $c = a (b - b_0)^{1/2}$ and $a = k_p / k_t^{1/2}$

Stefanovich and Karel (1982) studied the kinetics of the degradation of carotene using a model system composed of beta-carotene in microcrystalline cellulose by the two approaches described. They observed that neither one was really adequate to simulate the degradation of beta-carotene because they do not consider induction period, and they depend on the initial concentration of carotene as well as on the specific surface area of the support. At high concentrations of beta-carotene and low specific surface area, oxygen diffusion becomes a limiting factor.

During all these investigations on the kinetics of beta-carotene, the quantitation of beta-carotene was based on direct absorbance measurements at 436, 450 and 470 nm.

This method has its limitations which could contribute to the inadequacy of the investigated kinetics. Another approach to studying the degradation of beta-carotene would be through the monitoring of both beta-carotene loss (by means of an accurate method for beta-carotene) and volatile degradation products. The product formation could be related to beta-carotene loss.

In the presence of lipids, carotenoids have been reported to undergo coupled oxidation at rates dependant on the system. The kinetics of carotenoid degradation in the presence of lipids has not been investigated yet. Water activity has been observed to affect the rate of carotene degradation and its overall effect will depend on the composition of the system. In some cases, a change in the water content could mobilize pro- or antioxidant factors of the system or new sites on the solid matrix could be exposed due to swelling, resulting ultimately in different oxidation rates. The kinetic model of beta-carotene degradation as affected by lipids, water activity, oxygen, presence of anti- or pro-oxidants needs to be studied.

5. BREAKDOWN PRODUCTS FROM BETA-CAROTENE DEGRADATION

Knowledge of breakdown products of carotenoid origin would provide an understanding of the pathway by which carotenoids are oxidized in a food system. There are three

types of oxidation: chemical oxidation (a free radical initiator is added), thermal oxidation (free radicals are generated in the food system without the addition of any agents), and photosensitized oxidation (photoinitiation).

Chemical Oxidation

In the early structural studies on carotenoids, much valuable information was obtained by its partial degradation. By carefully controlled chromic acid oxidation of beta-carotene, Kuhn and Brockmann (1935) succeeded in isolating partial oxidation products such as beta-carotenone and the C₂₇-aldehyde. Degradation of the chain was observed to follow the initial opening of the beta end group. By partial permanganate oxidation of beta-carotene, Karrer et al. (1937) obtained polyene aldehydes apo-8'- and apo-12'-carotenal. Permanganate degraded the polyene chain leaving one ring intact. In both permanganate and chromic acid oxidations, alpha-carotene degrades from the beta-end to yield products isomeric to those obtained from beta-carotene (Moss and Weedon, 1976). During the incomplete monoperphthalate oxidation of beta-carotene, Seely and Meyer (1971) obtained beta-carotene 5,6-monoepoxide, beta-carotene 5,6,5',6'-diepoxide, beta-carotene 5,8-monoepoxide (mutatochrome) and beta-carotene 5,6, 5',8'-diepoxide (luteochrome). In the presence of metachloroperbenzoic acid, Marty and Berset (1986) observed that beta-carotene oxidized

into beta-carotene 5,6-monoepoxide, beta-carotene 5,8-monoepoxide and beta-carotene 5,6,5',8'-diepoxide.

Autoxidation

Hunter and Krakenberger (1946), examined the products formed by oxidation of beta-carotene in benzene by oxygen at 50°C. They observed that the course of oxidation of beta-carotene consisted of attack on a terminal double bond to give beta-carotene epoxide. This was converted in the presence of acid into mutatochrome which then underwent fission to yield semi beta-carotenone. A similar attack of the second beta-ionone ring led to the production of luteochrome, aurochrome (5,8,5',8'-diepoxy beta-carotene) and beta-carotenone. El-Tinay and Chichester (1970) investigated the reaction between beta-carotene and molecular oxygen in toluene at 60°C. The products of the reaction were beta-carotene 5,6-monoepoxide, beta-carotene 5,8-monoepoxide, beta-carotene 5,6,5',6'-diepoxide and beta-carotene 5,8,5',8'-diepoxide.

During the oxidation of beta-carotene in the dark, Isoe et al. (1969) identified dihydroactinidiolide and beta-ionone, both of them present in very low concentrations. When photooxidation of beta-carotene was carried out in benzene and methanol under irradiation by 30w fluorescent lamp, the composition and relative yield of oxidation products were parallel to that in the dark except that the degradation products were present at higher concentration

(Isoe et al., 1969). Additional studies (Isoe et al., 1972) on the exhaustive photooxidation of beta-carotene in benzene in the presence of trace amounts of water yielded in addition to dihydroactinidiolide and beta-ionone, 2-hydroxy-2,6,6-trimethylcyclohexanone, geronic acid and desoxy-xanthoxin.

Several studies have reported the formation of toluene, xylene, ionene and 2,6-dimethylnaphthalene as thermal degradation products of beta-carotene (Kuhn and Winterstein, 1932; Jones and Sharpe, 1948; Day and Erdman, 1963; Mader, 1964; Edmunds and Johnstone, 1965 and Schwieter et al. 1969). Alpha- and beta-ionone have been found during heating of beta-carotene in water at 100°C for 30 minutes and also in benzene at 188°C for 72 hours in the presence of air (La Roe and Shipley, 1970). Beta-cyclocitral, 5,6-epoxy beta-ionone and dihydroactinidiolide were identified during heating of beta-carotene at 190 and 220°C for 10 minutes in the presence of nitrogen or air (Schreier et al., 1979).

Marty and Berset (1986) identified beta-carotene 5,6-epoxide, beta-carotene 5,6,5',6'-diepoxide and beta-carotene 5,8-epoxide formed during heating of all-trans-beta-carotene in sealed glass tubes for two hours at 180°C. They also identified, after extrusion cooking (15 minutes at $180 \pm 2^\circ\text{C}$) of a model system consisting of corn starch impregnated with all-trans-beta-carotene: beta-carotene 5,6-epoxide, beta-carotene 5,6,5',6'-diepoxide, beta-carotene 5,8-epoxide and beta-carotene 5,6,5',8'-diepoxide. Beta 13-

apo-carotenone, beta 15-apo-carotenal and beta 14-apo-carotenal have been identified as thermal degradation products of beta-carotene during a simulated deodorization of palm oil (Ouyang et al., 1980).

Suyama et al. (1983) investigated the thermal oxidation of vitamin A palmitate in nonfat dry milk. During thermal oxidation in the dark, five hours at 100-110°C, the main degradation products were geronic acid, beta-cyclocitral, beta-ionone, 5,6-epoxy beta-ionone, dihydroactinidiolide and ionene in order of decreasing concentrations. By increasing the temperature to 150°C for two hours, there was an increase in the concentration of all oxidation products except beta-ionone and 5,6-epoxy beta-ionone. During the photooxidation of vitamin A palmitate at 100-110°C, Suyama et al. (1983) observed a very similar pattern of major volatiles to the one observed in the dark, the only difference being that the relative concentration of beta-cyclocitral and geronic acid were very close.

Photosensitized Oxidation

After an exhaustive oxidation of beta-carotene in the presence of chlorophyll and white light, Tsukida et al. (1969) found 5-hydroxy-8-ketocarotenoid, 8-hydroxy-mutatochrome, acetylenic hydroxy-ketocarotenoid and allenic hydroxy-carotenoid. Hasegawa et al. (1969) detected possible epoxides and hydroxy compounds after oxidation of beta-carotene sensitized by toluidine blue. Seely and Meyer

(1971) observed products of oxidation of beta-carotene photosensitized by hypericin in acetone to be mainly mutatochrome, and small amounts of aurochrome and other compounds absorbing in the violet and near U.V. regions of the spectrum.

Dihydroactinidiolide and a small amount of beta-ionone were isolated from products of the oxidation of beta-carotene in benzene and methanol under irradiation by 30 w fluorescent lamp in the presence of catalytic amounts of alkali and rose bengal (Isoe et al., 1969). Suyama et al. (1983), identified during the photosensitized oxidation (rose bengal) of Vitamin A palmitate in nonfat dry milk at room temperature beta-cyclocitral, beta-ionone, 5,6-epoxy beta-ionone, and dihydroactinidiolide.

Off Flavor and Beta-Carotene Degradation Products

Volatile degradation products of beta-carotene have been associated with food acceptability. Among volatile degradation products identified through the literature, the following have impact on flavor. Beta-ionone has been reported to have a violet flavor (Falconer et al, 1964), and hay like and floral flavor (Yamanish et al., 1980). 5,6-Epoxy beta-ionone has a sweet ionone-like flavor according to Ina and Eto (1972) and hay like and floral flavor according to Yamanishi (1981). Dihydroactinidiolide has been described as having a weak black tea aroma according to Ina and Sakato (1968), Ina et al. (1970) and Sakato et al.

(1972), a typical hay like flavor (Suyama et al., 1983) and also a peach and gardenia like flavor (Yamanishi, 1981). Alpha-ionone has a cedar wood and violet odor (Merck Index, 1983).

Vitamin A Activity of Beta-Carotene Degradation Products

The degradation of beta-carotene is also associated with pro-vitamin A activity loss. Among non volatile degradation products identified during beta-carotene degradation, beta-carotene 5,6-epoxide (21%), beta-carotene 5,6,5',6'-diepoxide (active), apo-8-carotenal (72 %) and apo-12-carotenal (120%) retain their provitamin A activity. Values in parentheses are provitamin A activity factors, compared to that of beta-carotene.

6. STABILITY OF BETA-CAROTENE IN MODEL SYSTEMS

The great difficulty of studying autoxidation of beta-carotene in impure systems is that the origin of isolated compounds cannot be determined and in such complex systems as food products there is no way of knowing where to look for products of oxidation. The most promising way to solve these problems is to investigate the mechanism and the general kinetic principles of the degradation of beta-carotene in a model system simulating the food. Model systems simulating dehydrated foods consist of pigment

supported on well-defined adsorbents and permit a detailed investigation of the degradation process.

Effect of Model System Adsorbent

The effect of the model system adsorbent on the rate of beta-carotene decolorization at 2% oxygen in the headspace was investigated by Goldman et al. (1983). It was observed that replacement of microcrystalline cellulose by magnesium oxide and aluminum oxide resulted in a significant increase in the rate of decolorization. The loss of 50% color occurred at 25, 10 and 1 days for microcrystalline cellulose, magnesium oxide and aluminum oxide, respectively. These supporting materials are arranged in order of increasing adsorption capability (Davies, 1976). Ramakrishnan and Francis (1979a) observed carotenoids to be more stable in starch systems than in microcrystalline cellulose systems.

Teixeira Neto et al. (1981) measured oxygen sorbed and/or entrapped by dehydrated products. Microcrystalline cellulose (Avicel) was observed to entrap $0.26 \pm 0.02 \mu\text{M}$ of oxygen per gram of support. The knowledge of adsorbed oxygen is very important as it remains available for oxidation even when headspace oxygen is removed by vacuum packing.

Effect of Water Activity

Chou and Breene (1972), Arya et al. (1979a), Ramakrishnan and Francis (1979a) and Goldman et al. (1983)

observed that increasing water activity increased the stability of beta-carotene in model systems. Various mechanisms have been suggested by which water reduces oxidation: (1) water attaches to sites on the surface, excluding oxygen from lipid materials (Salwin, 1959); (2) hydrogen bonding of hydroperoxides with water and metal catalyst inactivation by water (Quast et al., 1972); (3) reduction of free radical content by interaction with water (Labuza et al., 1970).

Contradictory results have also been reported. Baloch et al. (1977c) observed that carotenoid oxidation was lowest in samples that were stored at the monolayer water activity. The increase in carotenoid oxidation was greater at lower water activities than at higher water activities. They suggested that the increase in oxidation with increasing water activity could be due to the exposing of new sites on the solid matrix as a result of swelling of the matrix.

Table 2 summarizes the results of studies on the effect of water activity in model systems. Although rate constants are within the same range, these data are difficult to interpret. Baloch et al. (1977c) and Ramakrishnan and Francis (1979a) did not report the initial concentration of beta-carotene in the model system. Arya et al. (1979a) did not control storage temperature, allowing temperature fluctuations that could have significant effects on the degradation rate. Calculated beta-carotene degradation rate constants based on data by Ramakrishnan and Francis (1979a)

Table 2. First order rate constants for beta-carotene decolorization in model systems at several water activities.

References	Experimental Conditions		Rate Constants	BET
		% RH	k (hr ⁻¹)	
Chou and Breene, 1972	5 mg/g beta-carotene			
	20°C	0	3.7×10^{-3} (4.7×10^{-3})	24% RH
	Avicel	44	2.1×10^{-3} (3.1×10^{-3})	3.3% H ₂ O
Baloch et al., 1977a	? mg/g beta-carotene	11	28.7×10^{-3}	
	37°C	31	17.0×10^{-3} (21×10^{-3})	30% RH
	microcrystalline cellulose	51	18.7×10^{-3}	2.6% H ₂ O
Ramakrishnan and Francis, 1979a	? mg/g beta-carotene	dry	39.6 (4.2×10^{-3})	
	25°C	11	33.8 (3.9×10^{-3})	18% RH
	Avicel	23	27.0 (2.8×10^{-3})	2.8% H ₂ O
		52	19.2 (2.0×10^{-3})	
		75	16.7 (1.8×10^{-3})	
Arya et al., 1979a	1.7 mg/g beta-carotene	0	(5.8×10^{-3})	
	24 ± 8°C	22	(4.4×10^{-3})	
	microcrystalline cellulose	33	(3.4×10^{-3})	
		43	(2.6×10^{-3})	
		73	(2.3×10^{-3})	
Goldman et al., 1983	4 mg/g beta-carotene	0	(1.5×10^{-3})	
	35°C	33	(1.05×10^{-3})	
	2% O ₂	84	(0.78×10^{-3})	
	microcrystalline cellulose			

Values in parentheses are first order rate constants calculated from literature data.

are significantly different from reported values.

Baloch's assumption that carotenoid oxidation was lowest at the monolayer water activity does not seem to be valid. There does not seem to be any significant difference between beta-carotene degradation rate constant at 31 and 51 percent relative humidity. Monolayer water activity was calculated in three different studies according to the BET equation described by Salwin (1963). Monolayer water activity in each system was significantly different from the others and no correlation could be established with temperature, moisture content or beta-carotene concentration in the model system.

Since most of the methods used for carotenoid quantitation (Table 2) were based on reflectance or absorption at 449-500 nm, previous researchers were not measuring oxidation but decolorization. Budowski and Bondi (1960) and Ramakrishnan and Francis (1979a) observed that some oxidized products may also absorb at these wavelengths. In fact, by comparing the rate of carotenoid loss by TLC with the rate of absorbance loss by absorbance at 450 and 464 nm in studies by Hunter and Krakenberger (1946) and Ramakrishnan and Francis (1979a) in Table 3, the rate of carotenoid loss is higher than the rate of color loss.

Effect of Temperature

The effect of temperature on the degradation of beta-carotene in model systems is summarized in Table 1. As it is

Table 3. First order rate constants for the decolorization (absorbance) and for the loss of beta-carotene (TLC) during autoxidation of beta-carotene in arachis oil, benzene and avicel model systems.

References	Experimental Conditions		Rate Constants k (hr ⁻¹)	
			beta-carotene loss (TLC)	Color loss (Absorbance)
Hunter and Krakenberger, 1946	0.5 g/500g beta- carotene			
	50°C			(464 nm)
	arachis oil		11.0 x 10 ⁻³	7.6 x 10 ⁻³
	benzene		13 x 10 ⁻³	8.3 x 10 ⁻³
Ramakrishnan and Francis, 1979a	? mg/g beta-carotene			(450 nm)
	25°C	% RH		
	Avicel	0	57	39.6
		75	24	16.7

expected, an increase in temperature leads to increase in rate constants, however, rate constants observed do not agree well among experiments done at similar conditions. The activation energy obtained by Stefanovich and Karel (1982) for a temperature range of 60-80°C is much larger than those reported in the literature for lower temperature ranges. Finkel'stein et al. (1973) observed this same pattern in studies on the oxidation of beta-carotene in solid films. At approximately 27°C, they observed a sharp change in the temperature dependence of the rate, accompanied by an approximate two fold increase in the activation energy in the stationary section and concluded that such phenomena are usually caused by a change in the nature of the molecular mobility of the system as a result of phase transition. El-Tinay and Chichester (1970), however, obtained an activation energy of 10.20 kcal/mol for a temperature range of 60 to 90°C in the oxidation of beta-carotene in toluene (0.1 mg/ml).

Effect of Oxygen Pressure

A summary of studies investigating the effect of oxygen on beta-carotene decolorization in model systems is presented in Table 4. The influence of oxygen concentration in the headspace on decolorization rate was evident even at relatively low oxygen concentrations. Calculated rate constants, however, do not agree among experiments. Baloch et al. (1977c) observed an induction period not observed by

Table 4. First order rate constants and induction period for beta-carotene decolorization in model systems at several oxygen pressures.

References	Experimental Conditions		Rate Constant	
			k (hr ⁻¹)	Induction (hr)
Baloch et al., 1977a	? mg/g beta-carotene	100	17 x 10 ⁻³	19.2
	37°C	75	7.9 x 10 ⁻³	28.8
	31% RH	25	2.9 x 10 ⁻³	48.0
	microcrystalline cellulose	0	0.9 x 10 ⁻³	120.0
Teixeira Neto et al., 1981	.3 mg/g	1	1.0 x 10 ⁻³	
	37°C RH?	2	2.5 x 10 ⁻³	
	Avicel + NFDM			
Goldman et al., 1983	.4 mg/g	1	0.76 x 10 ^{-3*}	
	35°C	2	1.7 x 10 ⁻³	
	dry	10	5.9 x 10 ⁻³	
	microcrystalline cellulose	15	7.6 x 10 ⁻³	
		20	10 x 10 ⁻³	
		0	.096x 10 ⁻³	

* First order rate constant calculated from literature data.

others. The initial concentration of beta-carotene in the system was not reported.

Goldman et al. (1983) observed that shelf life or degradation time at 50% retention was 37, 25, 10, 7 and 5 days for samples stored at 1, 2, 10, 15 and 20% oxygen respectively. When oxygen was excluded from the headspace, beta-carotene deterioration was only 12% after 60 days of storage. Such a loss under nitrogen could be due to the presence of oxygen adsorbed or entrapped by the system. According to Teixeira Neto et al. (1981), adsorbed oxygen remains available for oxidation even when headspace oxygen is removed by vacuum packing. Therefore, the amount of oxygen adsorbed by a food is of importance in its storage stability. Teixeira Neto et al. (1981) included nonfat dry milk in the model system, incorporating, some pro- and antioxidant effects (the composition of the nonfat dry milk was not cited). Also, by adding nonfat dry milk to the microcrystalline cellulose, the amount of oxygen entrapped by the support was increased from 0.26 ± 0.02 to 0.54 ± 0.04 uM/g, increasing the oxygen available for oxidation. They also did not adjust system for water activity.

One interesting aspect of the decolorization of beta-carotene is the quantity of oxygen uptake during oxidation. Gagarina et al. (1970) studying beta-carotene oxidation in aromatic solvents (benzene and xylene) observed that the maximum rate of oxygen absorption exceeded the maximum rate of consumption of carotene by a factor of approximately 3.5.

They also observed that oxygen absorption continued for a long time after the disappearance of the initial beta-carotene. According to Goldman et al. (1983), for the oxidation of one mole of beta-carotene, eight moles of O_2 were required. Teixeira Neto et al. (1981) reported this same high ratio for the microcrystalline cellulose-nonfat dry milk system. The high oxygen consumption ratio was explained by the participation of oxygen in several simultaneous reactions, by the existence of a sequence of reaction in the mechanism, and also by the interaction of the intermediate products with oxygen. Teixeira Neto et al. (1981) tested this possibility by using different initial concentration of oxidation products, but results did not confirm the assumption. Elucidation of this phenomenon will require further work.

Effect of Free Radicals

Alekseev et al. (1968) investigated the oxidation of beta-carotene in solution (benzene and n-decane) at $50^\circ C$ in the presence of free radical initiators AIBN-azobisisobutyronitrile and dicyclohexylperoxydicarbonate-DHPC. Beta-carotene was observed to decompose in the presence of these compounds even in an atmosphere of an inert gas what was considered to be due to its high degree of unsaturation. El-Tinay and Chichester (1970) investigated the oxidation of beta-carotene in the presence of AIBN and NBS-N-bromosuccinimide. With catalytic amounts ($2 \times 10^{-4} M$)

of AIBN, the initial rate of loss of beta-carotene increased continuously with time. In the presence of NBS ($2 \times 10^{-4}M$), a specific reagent for the preferential production of allylic radicals, the rate constant for the oxidation was 6.5 times greater than the control.

Goldman et al. (1983) stored microcrystalline cellulose containing beta-carotene (1.8-2.2 mg/g dry CMC) and radical initiator AIBN (3 mg/g CMC) under nitrogen in the dark and observed that 90% of the initial pigment concentration decolorized in only 16 days. In an atmosphere of oxygen, the rate of oxidation of beta-carotene increased in the presence of free radicals. Regardless of the nature of the initiator, oxygen increased the rate of consumption of beta-carotene 2.5 to 3.5 fold in comparison with nitrogen (Alekseev et al., 1968).

Effect of Fatty Acids

Hunter and Krakenberger (1946) investigated the oxidation of beta-carotene in arachis oil (peanut oil) and in benzene. Beta-carotene degradation was observed to follow a first order reaction. Calculated rate constants from literature data are shown in Table 3.

A striking contrast in the rate of oxidation is evident and lower rates of carotenoid degradation in arachis oil were attributed to the presence of natural antioxidants and unsaturated fatty acids. The course of oxidation of beta-carotene by oxygen followed in arachis oil and in benzene

was observed by Hunter and Krakenberger (1946) to be similar, furnishing the same oxidation products. Fatty acids, themselves, particularly the unsaturated ones, undergo autoxidation at a rapid rate and produce considerable amounts of free radicals. The oxidative susceptibility of carotenoids might be influenced by the free radicals resulting from the fatty acid.

The existing literature relating fatty acid media and degradation of carotenoids can be divided into two categories. One group claimed that unsaturated fats increase the stability of carotenoids (Mitchell et al. 1954; Bickoff et al., 1955 and Savinov and Mikhailovania, 1958). The other group concluded that unsaturated fats accelerate the disappearance of carotenoids (Budowski and Bondi, 1960; Blain and Shearer, 1963 and Lime, 1969).

Ramakrishnan and Francis (1979a) investigated the degradation of beta-carotene in methyl esters of fatty acids and observed that the stability of beta-carotene in methyl esters of stearic, oleic, linoleic, and linolenic acids increased with increased unsaturation. They suggested that in the saturated fatty acid ester system, the free radicals (saturated peroxy radicals) are extremely unstable and very reactive. The free radicals have to choose between unreacted saturated fatty acid esters and beta-carotene. The carbon atom alpha to the terminal double bond of the pigment molecules are likely to be the most favored site in this system and all the available free radical may end up

abstracting hydrogen from beta-carotene. In the case of unsaturated fatty acid systems, they suggested that only a minor fraction of the available free radicals would eventually attack beta-carotene.

In a system of varied concentrations of methyl linoleate in hexane, Ramakrishnan and Francis (1979a) observed that as the concentration of unsaturated fat was increased, the extent of degradation of beta-carotene also increased. Baloch et al. (1977c), however, compared the activation energy for destruction of beta-carotene (10.3-12.8 Kcal/mol) with that for the oxidation of linoleic acid or its esters (15.2-17.2 Kcal/mole) in model system and suggested that carotenoid oxidation was more favored than the autoxidation of linoleic acid.

Arya et al. (1979a), investigating the effect of fatty acids esters on the stability of beta-carotene in isolated model systems (microcrystalline cellulose) at different water activities observed that methyl stearate and oleate enhanced the stability of beta-carotene whereas methyl linoleate stabilized beta-carotene during initial stages of slow oxidation after which it exhibited a strong pro-oxidant effect. They suggested that increased stability of beta-carotene in the presence of methyl esters of fatty acids could be due to the dilution effect whereas the prooxidant effect of highly unsaturated fatty acids like methyl linoleate could be due to their increased susceptibility

towards autoxidation and concomitantly increased concentration of peroxides which catalyze the destruction of beta-carotene.

Effect of Additives

Baloch et al. (1977c) investigated the effect of sulfur dioxide on the stability of beta-carotene in a microcrystalline cellulose model system and observed that sulfur dioxide not only increased the induction period of beta-carotene oxidation, but also reduced considerably the rate of beta-carotene destruction. The rate of beta-carotene destruction decreased rapidly with increasing concentration of SO₂ up to 2000 ppm, but further levels of SO₂ showed little increased protection. It was suggested that SO₂ protects beta-carotene in food because (i) it can react with beta-carotene through free radical mechanisms, (ii) it can react with peroxide radicals produced during processing and storage, and (iii) it can react with heavy metals. Sulfur dioxide, therefore, was considered to be very effective in inhibiting beta-carotene breakdown caused by molecular oxygen.

Arya et al. (1982) added amino acids (each 0.007 moles) to a microcrystalline cellulose system containing beta-carotene. It was observed that glycine, alanine, arginine, glutamine, glutamic acid and aspartic acid accelerated the rate of beta-carotene degradation while valine and threonine had practically no effect on beta-carotene stability.

Histidine, leucine, isoleucine and tryptophan, however, significantly stabilized beta-carotene. Arya et al. (1982) also investigated the effect of ascorbic acid (200 mg/ 85 mg beta-carotene) and quercetin (100 mg/ 85 mg beta-carotene) on the stability of carotenoids and observed that ascorbic acid exhibited only a slight stabilizing action while quercetin exhibited a strong protective effect.

Arya et al. (1979a) investigated the role of butylated hydroxy anisole (BHA) and propyl gallate (PG) on the stability of carotenoids and observed that both of them significantly lowered the rate of beta-carotene degradation. BHA was more effective in dry systems whereas PG was more effective above the monolayer region. Higher effectiveness of PG above the monolayer region was suggested to be due to its higher hydrophilicity compared with BHA and concomitant increased mobility in the system, increasing the chances of its interaction with the free radicals. Incorporation of fatty acid methyl esters along with BHA increased the protective action of BHA due to increase in its mobility.

7. STABILITY OF CAROTENOIDS IN DEHYDRATED CARROTS

Degradation of carotenoids has been reported to play an important role in determining the overall storage life of dehydrated carrots. Falconer et al. (1964) reported a significant correlation between the development of off-

flavors and color loss in freeze-dried carrot powder stored at 18°C. The off-flavors have been described as "violet and hay like aroma" (Walter et al., 1970) and are thought to be due to the autoxidation of carotenoids. As oxidation proceeds, a bitter soapy after-taste becomes apparent along with the violet like aroma. Tomkins et al. (1944) reported that off-flavors became noticeable and dehydrated product become unacceptable when 20-45% of the total carotenoid had been destroyed. Falconer et al. (1964) however observed that even when 5% color loss had occurred, off-flavor was detected. This violet-like aroma has been suggested to be due to the formation of beta-ionone (Tomkins et al., 1944 and Falconer et al., 1964), however it was observed that beta-ionone is not the only product of oxidation with hay-like flavor and can account for only part of the off-flavor of oxidized carrots (Falconer et al., 1964). Ayers et al. (1964) investigating off-flavor production in dehydrated carrots stored under oxygen, identified alpha-ionone, beta-ionone and 5,6-epoxy beta-ionone as substances with a violet like odor.

Effect of Water Activity

The effect of water activity on the stability of carotenoids in dehydrated carrots has been investigated by Tomkins et al. (1944) and Arya et al. (1979b). Tomkins et al. (1944), reported that the proportion of beta-carotene oxidized during storage of dehydrated carrots was higher at

8.2% than at 5.4% moisture content. According to Arya et al. (1979b), Tomioka et al. (1973) observed that carotenoids in freeze-dried carrots were more stable at 10% than at 0% moisture. Arya et al. (1979b), however, observed that carotenoids were relatively more stable in the range of 0.32 to 0.57 water activities (8.68 to 13.2% moisture content) the maximum stability being near 0.43 water activity (10% moisture content). Both above and below this level, the rate of carotenoid degradation increased significantly. The increase in the rate was greater at lower water activity than at higher water activity. Arya et al. (1979b) suggested that the increase in the rate of carotenoid degradation at higher water activities in freeze-dried carrots could be due to solubilization and concomitantly increased mobilization of catalysts present and exposure of new sites on the solid matrix as a result of swelling of the matrix.

In all these previous investigations, carotenoids were determined by measuring absorbance at 449 nm, therefore, total carotenoids were evaluated. Alpha- and beta-carotene constitute 20 and 70 percent, respectively, of the total carotenoid content in dehydrated carrots. No one has investigated the effect of water activity on individual carotenoids in dehydrated carrots.

In order to better analyze the effect of water activity on carotenoid stability in dehydrated carrots, first order rate constants were calculated by linear regression analysis for the data presented by Arya et al. (1979b) (Table 5).

Table 5. First order rate constants¹ for carotenoid degradation in dehydrated carrots at different water activities.

References	Experimental Conditions		Rate Constant*
		% RH	k (hr ⁻¹)
Arya et al., 1979b	Freeze dried carrots	0	11.0 x 10 ⁻⁴
	T = 24 ± 8°C	23	9.1 x 10 ⁻⁴
	O ₂	33	7.6 x 10 ⁻⁴
	t = 41 days	43	6.2 x 10 ⁻⁴
		57	6.2 x 10 ⁻⁴
		73	24.0 x 10 ⁻⁴

* First order rate constants calculated from literature data.

¹ Correlation for linear regression >.98

Based on the calculated rate constants, conclusions made by Arya et al. (1979b) are questionable: (i) with the exception of rate constants at 0% and 73% R.H., difference among rate constants might not be significant and would depend on number of replicates, standard deviation for the method of analysis; (ii) storage temperature fluctuations (16-32°C) could have significant effect on the rate of carotenoid degradation.

Effect of Oxygen Pressure

Stephens and McLemore (1969) stored carrot flakes packaged (i) in air and (ii) in an atmosphere of nitrogen (containing less than 2 percent oxygen) at 20°C. It was observed that carrot flakes packaged under nitrogen lost 17% in carotenoid content during the first month of storage, then carotenoid content remained constant for the next 23 months of storage. However, for the flakes packaged in air, at the end of the second month of storage a loss of 67% of carotenoids was observed and then carotenoids content remained at this level throughout the storage period.

Effect of Temperature

Stephens and McLemore (1969) followed carotenoid degradation in dehydrated carrot flakes packaged under Nitrogen at 0 and 20°C for a period of 24 months and observed the same pattern of carotenoid loss, and off-flavor development regardless of storage temperature.

Effect of Additives

Stephens and McLemore (1969) added salt (2% dry wt) during the preparation of carrot flakes by drum drying and saw no effect on the carrot flake quality during storage. Ellis et al. (1970) observed that NaCl acted as a pro-oxidant in dehydrated products. Speck et al. (1977) reported that salt treatment (37% of the total solids) before air drying significantly improved color, texture, flavor and carotenoid stability in dehydrated carrots. Arya et al. (1979b) soaked blanched carrots in 5% NaCl prior to drying and observed significant reduction on the rate of carotenoid degradation and on the rate of non-enzymatic browning as well as improved reconstitution characteristics of the dehydrated carrots.

Beneficial action of sulfites and antioxidants in stabilizing carotenoids in dehydrated vegetables has been reported by Nutting et al. (1970) and Roberts and McWeeny (1972). Tomkins et al. (1944) and Feinberg et al. (1964), however, reported that sulfite treatment did not significantly lower the rate of carotenoid destruction. Arya et al. (1979b) treated carrots with salt and sodium metabisulfite and observed improved stability of carotenoids in dehydrated carrots. Mohammad et al. (1973) observed that addition of sodium metabisulfite along with rice flour (0.05 and 2.5% respectively) proved beneficial in reducing beta-carotene losses during storage.

Weier and Stocking (1946) investigated the stability of carotene in dehydrated carrots impregnated with antioxidant and observed effects in order of increased stability: 0.01% NDGA (nordihydroguaiaretic acid), 0.1% pyrogallol, 0.1% ascorbic acid, 0.1% pyrogallol combined with 0.2% sodium metabisulfite, and 0.01% NDGA combined with 0.3% sodium metabisulfite. Mohammad et al. (1973) have reported that incorporation of BHA along with fats stabilizes but incorporation of BHA with propylene glycol had no effect on carotenoid stability in dehydrated carrot powder. Arya et al. (1979b) however, observed that incorporation of Embanox-6 (PG, BHA and citric acid in propylene glycol) significantly enhanced the stability of carotenoids and lipids in dehydrated carrots. It was also observed that incorporation of Embanox-6 along with salt and metabisulfite also tended to decrease carotenoid degradation and non-enzymatic browning.

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CHAPTER I

KINETICS OF BETA-CAROTENE OXIDATION. PART I DEVELOPMENT OF METHODOLOGY

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KINETICS OF BETA-CAROTENE OXIDATION. PART I DEVELOPMENT OF METHODOLOGY

ABSTRACT

Methodology was developed for the quantitation of beta-carotene concentration and for the identification of volatiles generated during oxidation of beta-carotene in a model system. An HPLC system was adapted and used with a gradient of acetone in hexane, to separate beta-carotene from its oxidation products. This technique recovers ninety six percent of the beta-carotene. Simple measurement of absorbance (the commonly used method) was shown to be inaccurate for monitoring beta-carotene loss as oxidation products also absorb at similar wavelengths.

Twenty-two volatiles were identified by GC/MS analysis of the headspace. Volatiles were collected by Tenax GC trap system and were derived from autoxidation at 20 and 80°C, photosensitized and chemical oxidation of beta-carotene. Oxidation products comprised mainly C₉, C₁₀, C₁₁ and C₁₃ compounds originated from cleavage of bonds 6-7, 7-8, 8-9 and 9-10 in the beta-carotene molecule. The major compounds

were alpha-isophorone, beta-cyclocitral, beta-ionone, 5,6-epoxy beta-ionone and dihydroactinidiolide. The last three have been reported as having hay like and violet off-flavors, which can reduce food product acceptability.

INTRODUCTION

The oxidation of beta-carotene has some commercial importance. It is desirable in the production of tea and tobacco as it plays an important role in their flavor and aroma development (Walhberg et al., 1977). In other foods such as dehydrated vegetables, however, oxidation of beta-carotene can deteriorate the attractive color of foods, their nutritive value and flavor, leading to unacceptability and reduced storage life (Archer and Tannenbaum, 1979). Accurate monitoring of beta-carotene in food systems would lead to improved information on the effects of storage on beta-carotene loss, to accurate prediction of vitamin content and to prediction of product acceptability.

Several attempts have been made to elucidate the mechanism for the oxidative degradation of beta-carotene (Alekseev et al., 1968; Gagarina et al., 1970; Chou and Breene, 1972; Finkel'stein et al., 1973, 1974; Arya et al., 1979; Ramakrishnan and Francis, 1979; Goldman et al., 1983). However, there is no consensus on what the degradation mechanisms are. In most of these investigations, the methodology used for determination of beta-carotene was not appropriate.

Beta-carotene analysis.

There are many references for beta-carotene determination (Parrish, 1977 and De Ritter and Purcel, 1981)

from simple direct spectral measurement to meticulous isolation. A mixture of beta-carotene with its degradation products is so complex that separation methods such TLC, open column chromatography and/or HPLC must be used for reliable quantitation. The studies of the mechanism for beta-carotene degradation usually measured spectral absorbance at 450 nm.

The quantitative determination of beta-carotene is very difficult. Beta-carotene is readily subject to isomerization and decomposition and requires special care to minimize its exposure to air, light or reacting chemicals (De Ritter and Purcell, 1981). Beta-carotene oxidizes to form several non-volatile degradation products such as beta-carotenone (Kuhn and Brockman, 1985), apo-8'- and apo-12'-carotenal (Karrer et al., 1937), 8-hydroxy mutatochrome (Tsukida et al., 1968), beta-carotene 5,6-epoxide, beta-carotene 5,8-epoxide and beta-carotene 5,6,5',8'-diepoxide (Hunter and Krakenberger, 1946; El-Tinay and Chichester, 1970; Seely and Meyer, 1971 and Marty and Berset, 1986) and beta-carotene 5,8,5',8'-diepoxide (Seely and Meyer, 1971). Most of these beta-carotene degradation products absorb in the same region in the visible spectra as beta-carotene does, interfering with its measurement.

Volatile degradation products.

Beta-carotene has two symmetrical ends connected by a chain containing carbon-carbon double bonds. There are a

number of sites at which oxygen can attack and degrade this molecule. Attacks between bonds 6-7, 7-8, 8-9 and 9-10 (or 6'-7', 7'-8', 8'-9' and 9'-10') result in end products which are volatile at moderate temperatures. Figure 1 shows beta-carotene with the bonds labelled. Attack may occur at higher bonds (> 9-10, 9'-10') along the chain, but these products are not volatile and therefore, may not contribute to off-flavors. Presumably, two volatile products may be produced from one beta-carotene molecule by cleavage near both ends of the molecule. There can be a number of possible degradation products resulting from the cleavage of one bond. Some of these products are shown in Figure 2, along with the numbering system used in this paper.

Several volatile degradation products have been identified (Table 1). Beta-ionone and dihydroactinidiolide (Isoe et al., 1969), geronic acid, 2-hydroxy 2,6,6-trimethyl cyclohexanone and desoxyxanthin (Isoe et al., 1972) were identified from photosensitized oxidation of beta-carotene. During thermal degradation, products identified included toluene, xylene, ionene and 2,6-dimethyl naphthalene (Kuhn and Winterstein, 1932; Jones and Sharpe, 1948; Day and Erdman, 1963; Mader, 1964; Edmund and Johnstone, 1965; Schwieter et al., 1969; Byers, 1983), alpha-ionone and beta-ionone (La Roe and Shipley, 1970), beta-cyclocitral, 5,6-epoxy beta-ionone and dihydroactinidiolide (Schreier et al., 1979). Oxygenated products originate from oxygen attack to bonds 6-7, 7-8, 8-9 and 9-10. Toluene, xylene and 2,6-

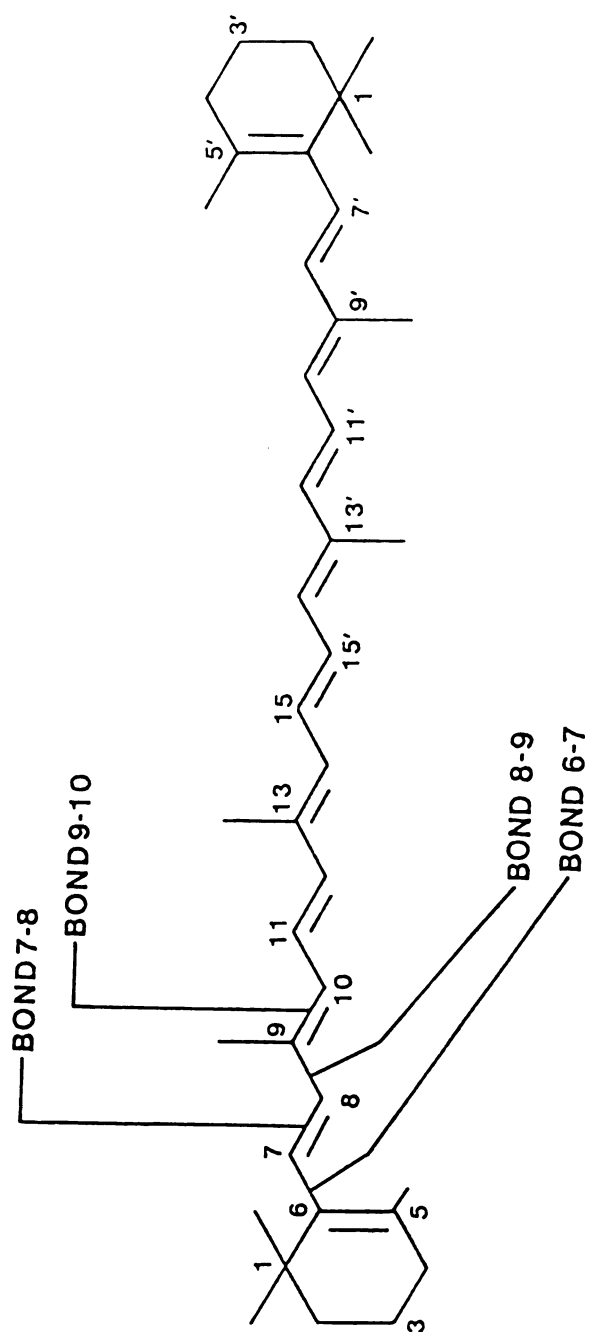
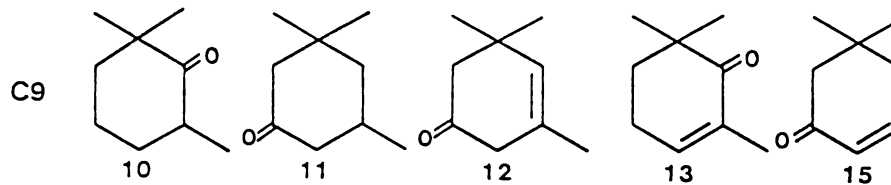
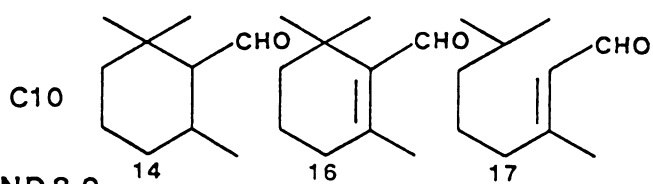


Figure 1. Bond location of oxygen attack resulting volatile degradation products from beta-carotene.

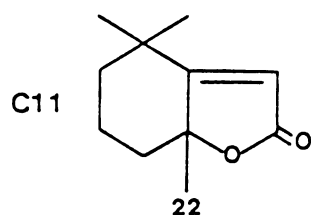
BOND 6-7



BOND 7-8



BOND 8-9



BOND 9-10

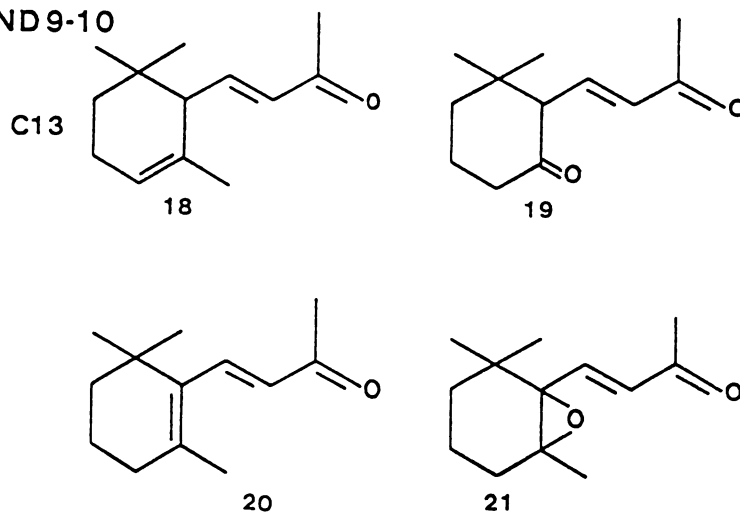


Figure 2. Beta-carotene volatile oxidation products categorized according to the bond they have originated from (Compounds numbered according to Table 2).

Table 1. Previously identified volatile products from beta-carotene degradation.

Name	Bond	Investigators
toluene		Day and Erdman, 1963 Mader, 1964 Edmund and Johnstone, 1965 Schwieter et al., 1969
xylene		"
ionene		"
2,6-dimethyl naphthalene		"
2-OH-2,6,6-trimethyl cyclohexanone	6-7	Isoe et al., 1969
beta- cyclocitral	7-8	Schreier et al., 1979
geronic acid	7-8	Isoe et al., 1972
dihydro actinidiolide	8-9	Isoe et al., 1969 Schreier et al., 1979
alpha-ionone	9-10	La Roe and Shipley, 1970
beta-ionone	9-10	La Roe and Shipley, 1970 Isoe et al., 1969.
5,6-epoxy beta- ionone	9-10	Schreier et al., 1979

dimethylnaphthalene are formed by cyclization and elimination reactions through a four membered ring intermediate.

Volatile degradation products are relatively easy to monitor. If a good correlation could be established between beta-carotene loss and volatile formation, the measurement of volatile formation could be used as an index of beta-carotene oxidation.

The objective of this study was to develop improved procedures for the determination of beta-carotene in a model system and to identify volatile beta-carotene oxidative decomposition products. These procedures can then be used to interpret experiments designed to show the mechanisms of degradation. For comparative purpose, volatile decomposition products from photosensitized and chemical oxidations were also studied.

EXPERIMENTAL PROCEDURES

Materials.

Synthetic all-trans-beta-carotene (Sigma Chemicals, St. Louis, MO) was purified by column chromatography using alumina as adsorbent. The purity was tested by HPLC. All solvents used for extraction were glass distilled, and the solvents used for HPLC were UV spectroscopic grade. Volatile standards were purchased from Sigma Chemicals (St. Louis, MO), Aldrich Chemicals (Milwaukee, WI) and ICN Chemicals

(Plainview, NY). Beta-isophorone (Mousseron-Canet et al., 1967), beta-cyclocitral (Suyama et al., 1983), 5,6-epoxy beta-ionone, dihydroactinidiolide (Isoe et al., 1972), were synthesized.

Methods of oxidation of beta-carotene.

Oxidation of beta-carotene was carried out under four different conditions. In each case, headspace volatiles were collected for identification and samples of the solid were analysed for beta-carotene.

(i) all-trans-beta-carotene (40 mg) impregnated in 40 g of microcrystalline cellulose-Avicel (FMC Corporation, Philadelphia, PA) (Chou and Breene, 1972; Baloch et al., 1977; Ramakrishnan and Francis, 1979; Arya et al., 1979 and Stefanovich and Karel, 1982) was placed in a 500 ml round bottomed distillation flask covered with aluminum foil and oxidized under 99.8% pure oxygen stream of 10 mL/min (Michigan Welding Supply, Lansing, MI), at $20 \pm 1^\circ\text{C}$.

(ii) The reaction conditions were the same as described in method i, except that the oxidation was carried out at $80 \pm 1^\circ\text{C}$.

(iii) A solution of 100 mg of all-trans-beta-carotene and 2 mg of rose bengal (Suyama et al., 1983) in 100 ml of absolute ethanol was placed in a 500 ml round bottomed distillation flask. The photosensitized oxidation was carried out at $20 \pm 1^\circ\text{C}$ by illuminating with a 20 W fluorescent lamp (soft white F20T12.SW, General Electric,

Cleveland, OH) at a distance of 5 cm from the reaction vessel with oxygen bubbling at a rate of 10 ml/min.

(iv) Metachloroperbenzoic acid (Aldrich Chemicals, Milwaukee, WI) was added to all-trans-beta-carotene (ratio of 0.2 mole/l mole) in methylene chloride solution. The solution was introduced into a flask topped with a calcium chloride guard and immersed in an ethanol bath at $0 \pm 1^\circ\text{C}$ with magnetic stirring (Marty and Berset, 1986).

Analysis of beta-carotene.

Beta-carotene was extracted with five 25 ml portions of acetone:petroleum ether (50:50) by vigorous vortexing, followed by centrifugation at 650 rpm for two minutes. The extract was washed free of acetone with water, filtered through anhydrous sodium sulfate, and concentrated to 10 ml.

UV-visible absorbance spectra were taken on a Perkin-Elmer Lambda 4B UV/Vis C099-1301 spectrophotometer attached to an Epson FX-80 printer. The samples were in petroleum ether. The wavelength range was 300 to 600 nm.

High Pressure Liquid Chromatography analysis of beta-carotene was performed on a M6000A Waters Associates Liquid Chromatograph attached to a Waters model 450 Variable Wavelength detector operating at 450 nm and an Omniscribe recorder. The column was a 30 x 0.39 cm 10 microns Micro-Porasil, Waters Associates, Inc., Milford, MA. A gradient of acetone in hexane (0.5, 2, 5, 8 and 15 % per 12 minutes each) was used at a flow rate of 1.5 ml/min. Beta-carotene

concentration was calculated by the regression line of the standard curve of beta-carotene correlating concentration and peak area ($y = 0.0052x + 0.00686$; $r^2 = 0.9981$).

Volatile degradation products.

The volatiles formed by the oxidation of beta-carotene were collected by a Tenax GC trap system (a modification of the method of Simon et al., 1980) using undecane as an internal standard (Figure 3). A 30^g sample was weighed into a 250 ml round bottom flask fitted to a 24/40 Vigreux distillation column connected to a glass elbow joint. A Tenax GC collection trap (0.2g Tenax GC, 80/100 mesh, Ohio Valley Specialty Chemicals, Marietta, OH. in a 9.0 mm x 0.6 mm glass tube) was placed in between the elbow and vacuum pump. Traps were held in contact with the elbow with Tygon tubing. Samples were held at 60°C for 1.5 hr at 18 inches of Hg. Volatiles were eluted from the Tenax GC trap by back flushing the trap with two 2 ml volumes of isopentane followed by centrifugation at 650 rpm for 2 minutes. As a control, volatiles were also collected for the purified all-trans-beta-carotene.

Separation of the volatile degradation products was performed on a Hewlett Packard 5890 Gas Chromatograph (Palo Alto, CA) with a Flame Ionization Detector and a Heliflex Bonded FSOT Superox polyethylene glycol capillary column (30 m x 0.25 mm) from Alltech Associates, Inc., Deerfield, IL. The temperature program was 30°C for 5 minutes, a rate of

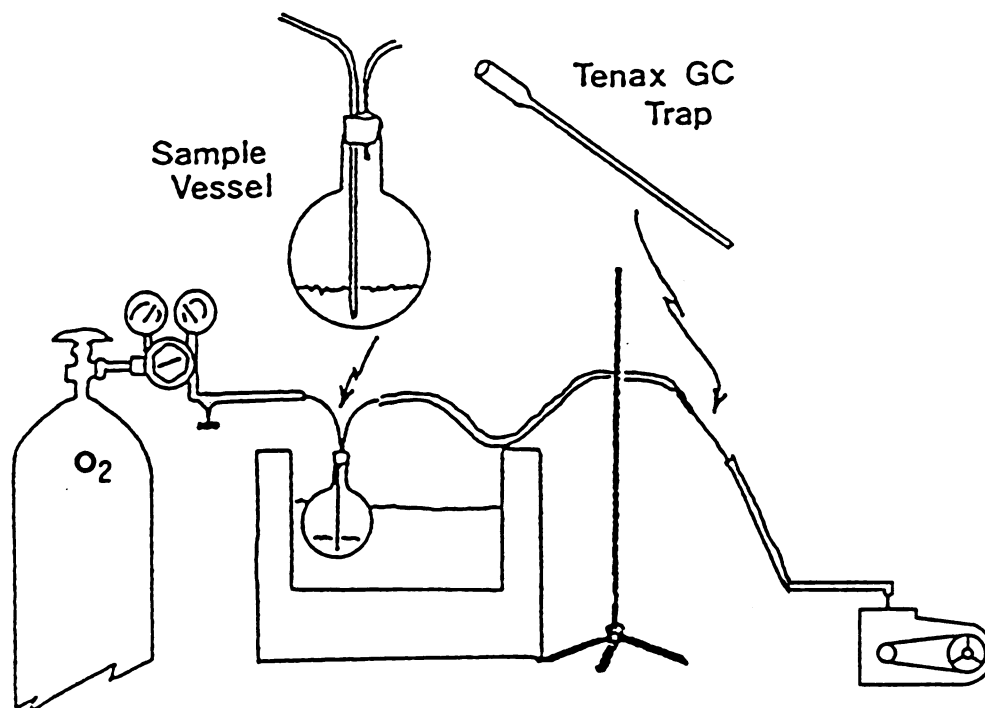


Figure 3. Apparatus used for the autoxidation of beta-carotene and collection of volatiles.

2°C per minute up to 180°C and then holding for 10 minutes. The split ratio was of 1:17. Injection temperature 200°C; detector temperature 275°C; carrier gas Helium at 75 psi.

The system used for GC/MS analysis of the volatile degradation products was a Nermag R-10-10C Mass Spectrometer interfaced with a Delsi splitless capillary GC and a bonded phase Supelcowax 10 (60m x 0.25 um) from Supelco, Inc., Bellefonte, PA. The temperature program was 40°C for 1 minute to 180°C at 2°C/min. holding at 180°C for 40 min. The instrument was operated in the electron impact mode at an ionization voltage of 70 eV. The ion source temperature was 200°C. A scan of 1 s. was used over a mass range of 40-250 atomic mass units.

Oxidation of primary volatile oxidation products.

The primary oxidations products of beta-carotene could undergo further transformations, oxidations, reductions and cyclizations (Enzell, 1981). In order to test if oxidation products are formed from a C₁₃ precursor such as beta-ionone, beta-ionone was oxidized (i) under a stream of oxygen at 20 ± 1 and 80 ± 1°C in the dark and (ii) under a stream of oxygen in the presence of rose bengal at 5 cm from a 20 w fluorescent lamp.

RESULTS AND DISCUSSION

Analysis of beta-carotene.

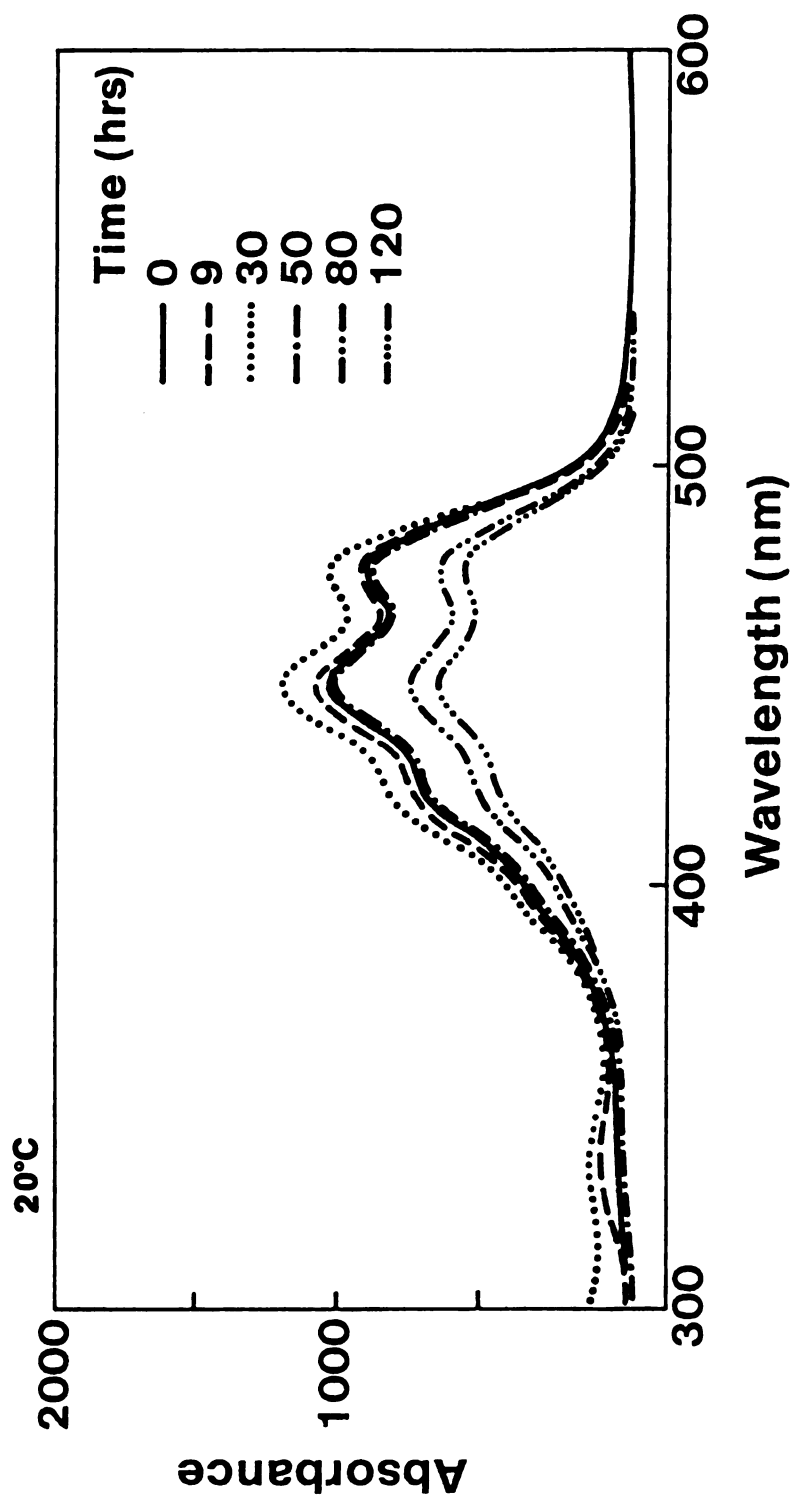
UV-Visible absorption spectra.

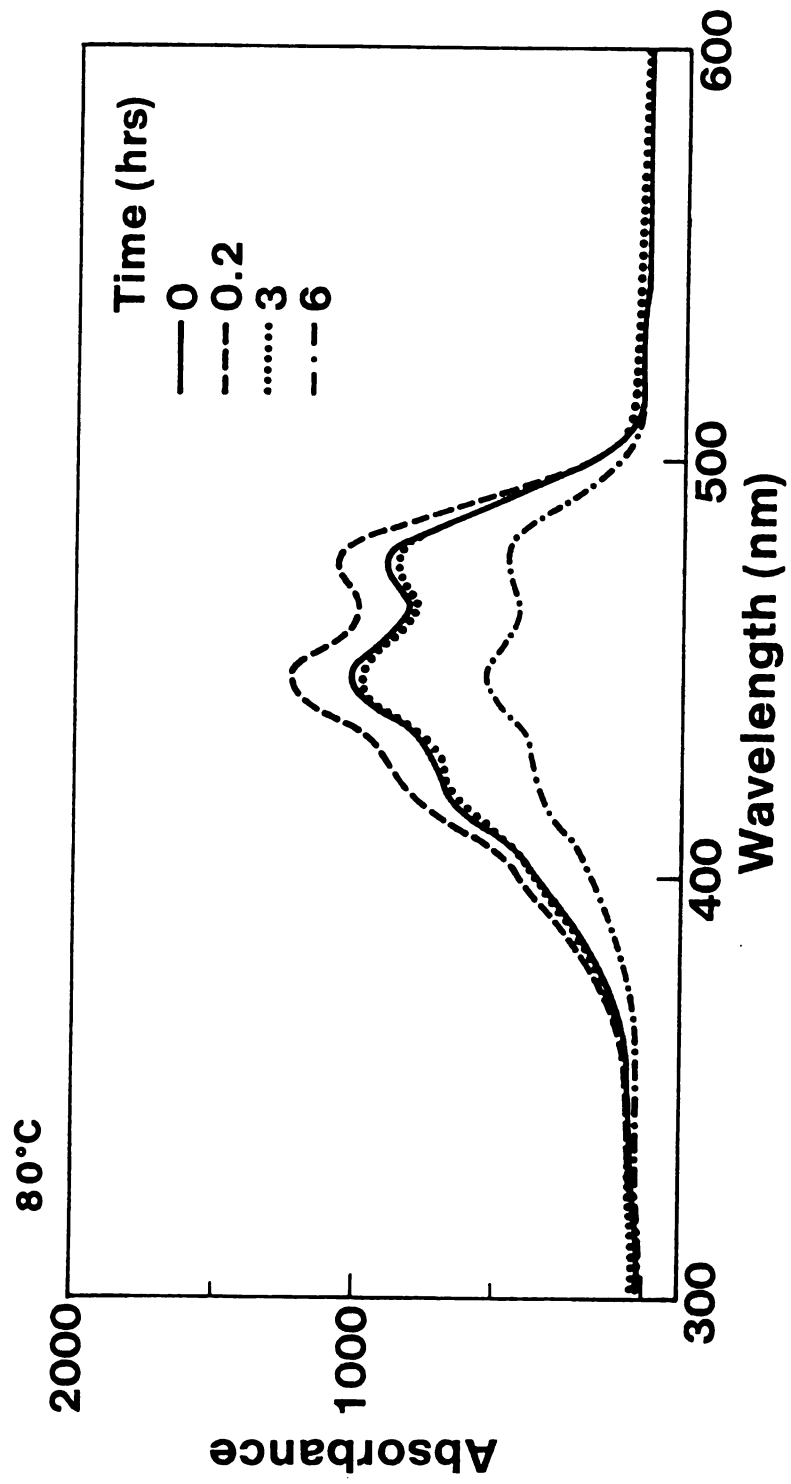
Figures 4 show the change in the UV-visible absorption spectra of beta-carotene caused by the different oxidation treatments. In every type of oxidation investigated, there was a significant increase in the apparent absorbance throughout the entire spectra before the absorption finally decreased. This same behavior has been observed by Hunter and Krakenberger (1946) during oxidation of beta-carotene in peanut oil and by Seely and Meyer (1971) during oxidation of beta-carotene photosensitized by hypericin.

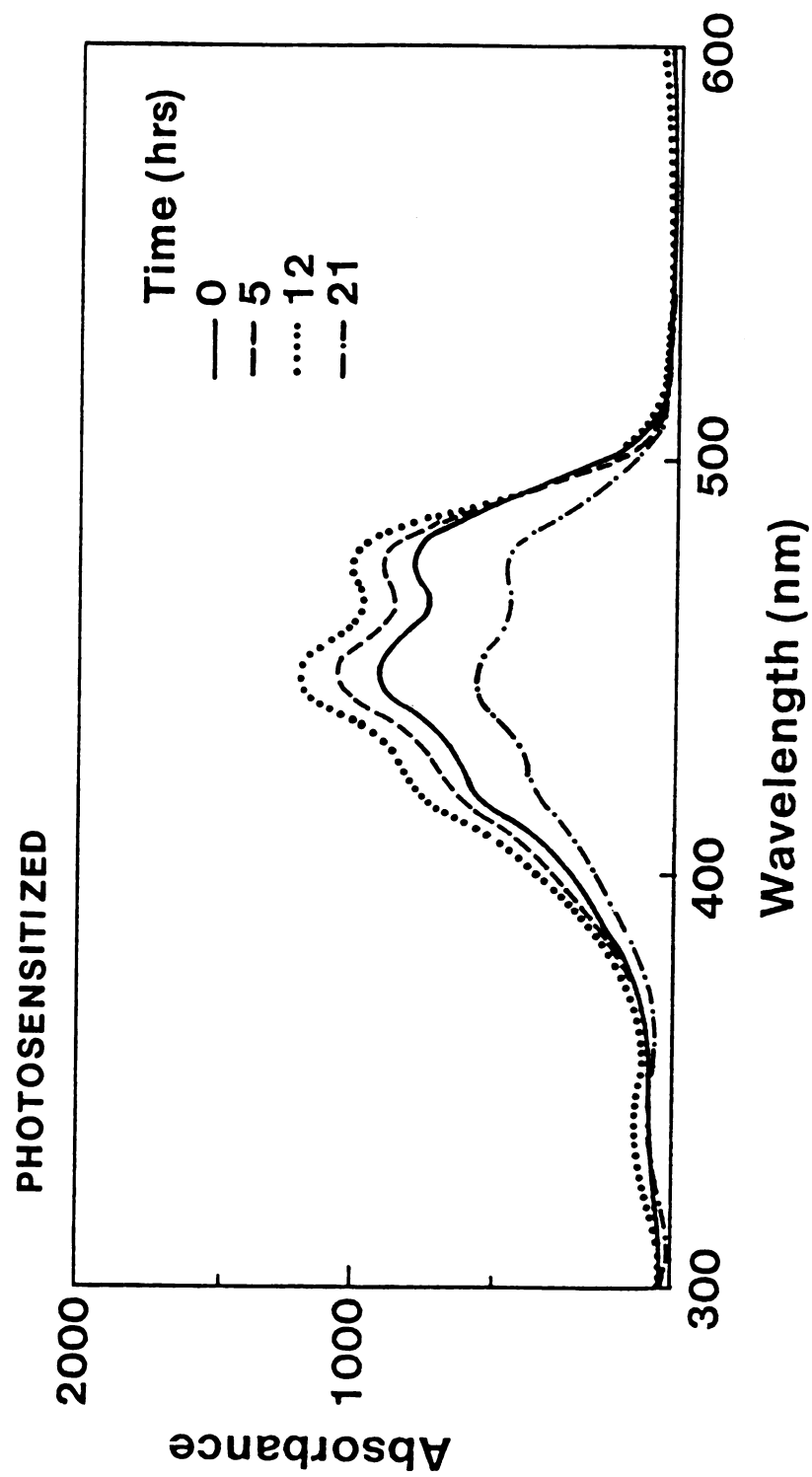
Potential for absorbance wavelength shifts.

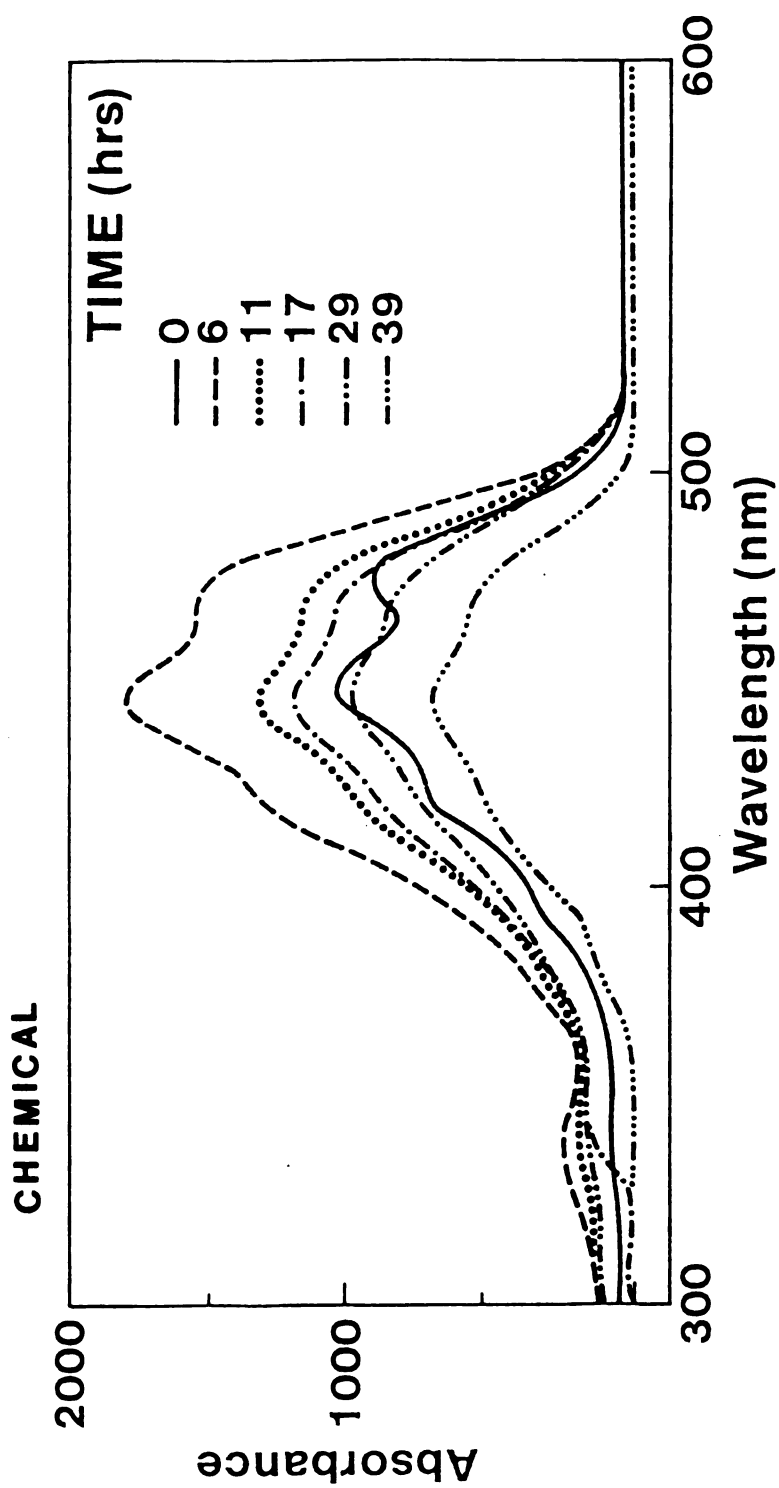
No shift in wavelength of the main absorption band (450 nm), or change in the overall shape of the spectra was observed except for the metachloroperbenzoic acid oxidation. According to Schwieter et al. (1969), 5,6-epoxy and 5,8-epoxy substituents on the beta-carotene molecule, as well as trans --> cis isomerization could cause a shift to shorter wavelengths of respectively 8, 20 and 4 nm (with respect to 450 nm). Addition of a first and second carbonyl group to the aliphatic chain or ring would cause a bathochromic shift of respectively 28, 1 to 7, 7, 5 to 9 nm. Trans --> cis isomerization was observed in every type of oxidation, based

Figure 4. UV-visible absorbance spectra of beta-carotene (in petroleum ether) and its degradation products at various times, during: autoxidation at 20 and 80°C, photosensitized and metachloroperbenzoic acid oxidation.









on the presence of a cis peak at 340 nm, however isomerization did not happen to a great extent since the intensity of the peak was low. The shift of the absorption maxima to shorter wavelengths during the metachloroperbenzoic acid oxidation, could be due to the predominance of an epoxy over ketone or aldehyde substituent in the degradation products. However, the accuracy and reproducibility of this spectrophotometric method are not high enough to monitor these shifts.

Increase in absorbance with degradation.

The increase in the absorption spectra could be due to the higher molar extinction coefficients of the degradation products (due to substituents functional groups) compared to that of all-trans-beta-carotene (Fopen, 1971). It is also possible that during beta-carotene degradation, breakdown of the molecule would generate an increased number of molecules and therefore an increased molar concentration.

Normal phase HPLC.

The absorbance spectra at 450 nm in Figure 4 demonstrate that absorbance does not measure beta-carotene loss alone. Therefore, an HPLC method was developed to separate beta-carotene from its degradation products and can be used to follow the loss of beta-carotene in model systems. Beta-carotene eluted within 3 minutes. According to the HPLC pattern observed for samples oxidized by the

different methods, beta-carotene was not the only compound absorbing at 450 nm (Figure 5). These compounds show polarity very similar to that of beta-carotene. During autoxidation at 80°C (at 0.2 hr) only $58.3 \pm 0.5\%$ of the absorbance at 450 nm was due to beta-carotene. Non-volatile degradation products were not identified as they were not the focus of this investigation; however, it should be pursued in further research.

Figure 6 shows beta-carotene concentration as a function of time for the four oxidation methods. In contrast with Figure 4, in which absorbance at 450 nm increases and then decreases, the HPLC method shows that beta-carotene concentration decreases monotonically as oxidation proceeds. The data in Figure 6 show that oxidation of beta-carotene occurs at a higher rate as temperature increases. This method clearly gives a better measurement of beta-carotene loss than the absorbance method of Figure 4.

Volatile products from oxidation of beta-carotene.

Identification of volatile products.

Results of this investigation show that a considerable number of volatile compounds are formed from beta-carotene oxidation. Table 2 lists the components identified in this study, their chemical formula and their Kovats Indices. The Kovats index describes the retention behavior of the volatile relative to that of the n-paraffin hydrocarbons. Several other volatile degradation products were also

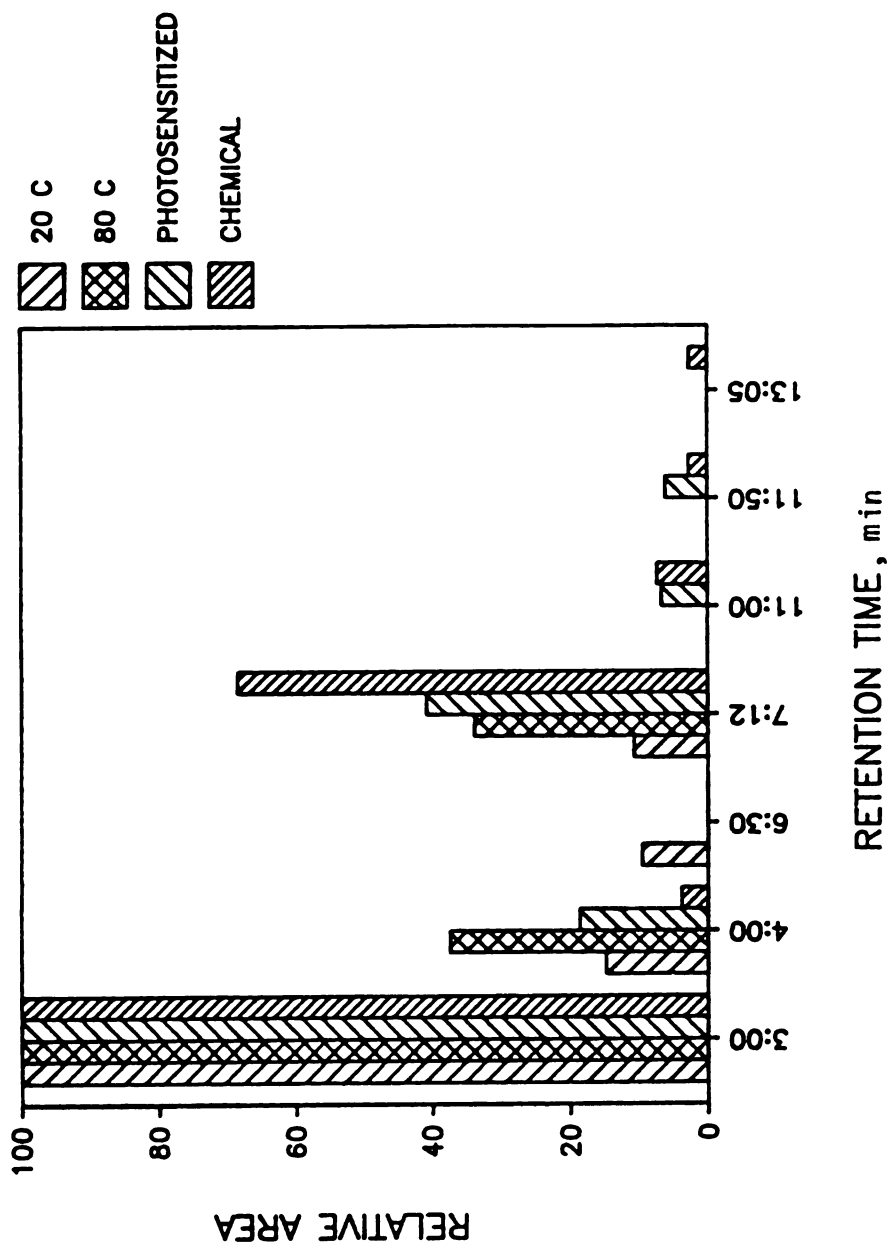


Figure 5. Contribution of non-volatile compounds separated by HPLC, to the absorbance at 450 nm at the time of maximum absorbance during autooxidation at 20 and 80°C, photosensitized and chemical oxidation of beta-carotene. Beta-carotene: 3 min.

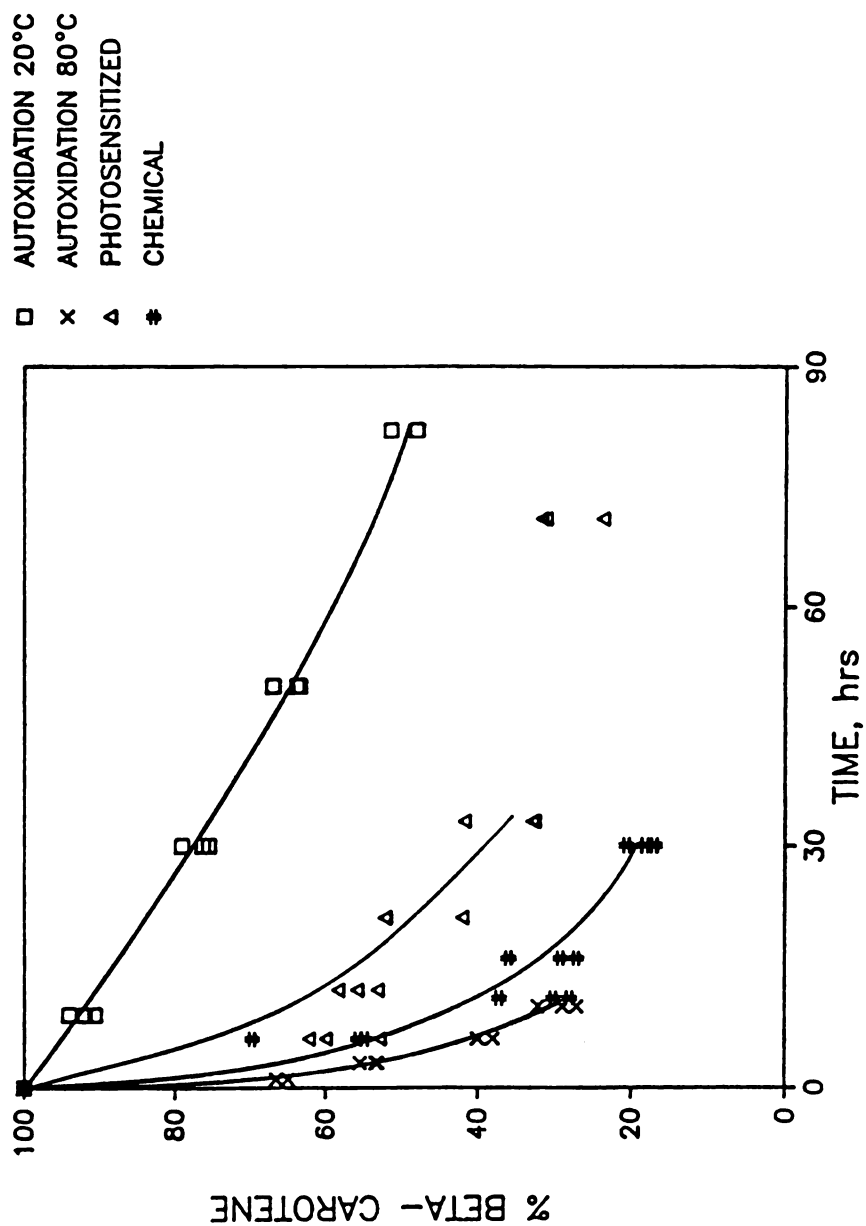


Figure 6. Beta-carotene concentration (measured by normal phase HPLC) as a function of time during autoxidation at 20 and 80°C, photosensitized and chemical oxidation.

Table 2. Volatile compounds identified^a during oxidation of beta-carotene, their formula, Kovax indices^b and bond they originate from.

Peak #	Bond	Compound	Formula	Kovats Index ^b
1		benzene	C ₆ H ₆	779
2		toluene	C ₇ H ₈	1018
3		p-xylene	C ₈ H ₁₀	1142
4		m-xylene	C ₈ H ₁₀	1147
5		o-xylene	C ₈ H ₁₀	1191
6		methyl ethyl benzene*	C ₉ H ₁₂	1193
7		isobutyl benzene*	C ₁₀ H ₁₄	1196
8		1,6-decahydronaphthalene*	C ₁₂ H ₂₂	1203
9		trimethyl benzene	C ₉ H ₁₂	1250
10	6-7	2,2,6-trimethyl cyclohexanone	C ₉ H ₁₆ O	1280
11	6-7	3,3,5-trimethyl cyclohexanone	C ₉ H ₁₆ O	1335
12	6-7	beta-isophorone	C ₉ H ₁₄ O	1353
13	6-7	2,2,6-trimethyl cyclohexenone	C ₉ H ₁₄ O	1374
15	6-7	alpha-isophorone	C ₉ H ₁₄ O	1546
14	7-8	2,2,6-trimethyl cyclohexane 1-carboxaldehyde	C ₁₀ H ₁₈ O	1497
16	7-8	beta-cyclocitral	C ₁₀ H ₁₆ O	1574
17	7-8	neral	C ₁₀ H ₁₆ O	1661
22	8-9	dihydroactinidiolide	C ₁₁ H ₁₆ O ₂	2250
18	9-10	alpha-ionone	C ₁₃ H ₂₀ O	1833
19	9-10	gamma-ionone	C ₁₃ H ₂₀ O	1882
20	9-10	beta-ionone	C ₁₃ H ₂₀ O	1918
21	9-10	5,6-epoxy beta-ionone	C ₁₃ H ₂₀ O ₂	1941

^a Experimental details are given in the text.

^b Kovats index was determined on the bonded FSOT Superox Polyethylene Glycol capillary column at conditions described in the text.

* The most reasonable formula postulated by M⁺ values and spectra pattern compared to NIH library of spectral data.

present, however their identification was not possible. The compounds identified represent approximately 90% of the total observed peak areas by Gas Chromatography.

Several aromatic and partially aromatic compounds were identified in the volatile fraction, and could also be found in unoxidized beta-carotene.

As shown in Table 2, the components of the oxidation of beta-carotene are mainly compounds retaining the intact ionone ring (aldehydes and ketones) and result from oxidative cleavage of the polyene chain. 5,6-Epoxy beta-ionone, dihydroactinidiolide, beta-ionone, alpha-isophorone, beta-cyclocitral, 2,2,6-trimethyl cyclohexane 1-carboxaldehyde comprise approximately 70-80% of the total volatile fraction. Alpha-isophorone (3,5,5-trimethyl 2-cyclohexenone), beta-cyclocitral (2,2,6-trimethyl 1-cyclohexene carboxaldehyde), and beta-ionone (2,6,6-trimethyl 1-cyclohexen-1-yl 3-buten-2-one) may be formed from the cleavage of the 6-7, 7-8 and 9-10 bonds, respectively. 5,6-Epoxy beta-ionone (4-(2,2,6-trimethyl 7-oxabicyclo 4,1,0-hept 1-yl) 3-buten 2-one) may be formed via cleavage of the 9-10 bond of 5,6-epoxy beta-carotene. Dihydroactinidiolide (5,6,7,7a-tetrahydro (4,4,7a-trimethyl benzofuranone) may be formed by cleavage of the 8-9 bond (Enzell, 1981), via 5,8-epoxides or via 5,6-epoxides which can rearrange to 5,8-epoxides (Wahlberg et al., 1977).

Oxidation of primary volatile oxidation products.

During autoxidation of beta-ionone, 5,6-epoxy beta-ionone was the major product until half of the beta-ionone had been consumed and then other degradation products started being formed, mostly low volatility. This same pattern was observed for both 20 and 80°C autoxidation except that at 80°C the rate was much higher.

Dihydroactinidiolide was not formed from the autoxidation of beta-ionone even after exhaustive autoxidation.

During photosensitized oxidation with rose bengal, beta-ionone oxidized mainly into dihydroactinidiolide and into 5,6-epoxy beta-ionone and beta-cyclocitral to a lesser extent.

Effect of oxidation method on volatile degradation products.

Table 3 summarizes the relative concentrations of identified volatiles, from unoxidized, purified and HPLC tested beta-carotene and from the different types of oxidation of beta-carotene, collected when 50% of the original beta-carotene had been oxidized. Similar degradation products were found in the different types of oxidation, however, their relative concentration varied. Beta-cyclocitral was the major component followed by 2,2,6-trimethyl cyclohexane carboxaldehyde in unoxidized beta-carotene. Beta-ionone was also observed but in smaller concentration. These compounds are all products of oxidative degradation of beta-carotene and their presence shows how

Table 3. Relative concentrations* of volatiles under various oxidation conditions^a.

Peak				Method ^a			
#	Bond	Compound	Control ^b	1	2	3	4
15	6-7	alpha-isophorone	0	11	0	0	0
14	7-8	2,2,6 trimethyl cyclohexane carboxaldehyde	67	100	1	15	35
16	7-8	beta-cyclocitral	100	16	0	9	48
17	7-8	neral	0	0	0	7	10
22	8-9	dihydro- actinidiolide	0	45	100	46	65
18	9-10	alpha-ionone	0	0	0	9	8
20	9-10	beta-ionone	15	13	7	100	100
21	9-10	5,6-epoxy beta- ionone	0	82	53	53	39

* The peak area of highest intensity was assigned a value of 100.

^a Experimental details are given in the text.

^b Volatile products associated with unoxidized beta-carotene.

labile beta-carotene is.

During autoxidation of beta-carotene at 20°C, 2,2,6-trimethyl cyclohexane carboxaldehyde was the major oxidation product, followed by 5,6-epoxy beta-ionone and dihydroactinidiolide. Bonds 9-10 and 7-8 were the major sites of oxygen attack. At this stage of oxidation, the concentration of 5,6-epoxy beta-ionone was higher relative to that of beta-ionone and could reflect simultaneous oxygen attack to bonds 9-10 and 5-6, as well as the oxidation of beta-ionone itself into 5,6-epoxy beta-ionone. Attack of oxygen to bond 8-9 was also observed leading to the formation of dihydroactinidiolide.

At 80°C, oxidation of beta-carotene led to mainly two degradation products: 5,6-epoxy beta-ionone and dihydroactinidiolide. The relative concentration of dihydroactinidiolide increased sharply as compared to 20°C.

During photosensitized oxidation of beta-carotene, beta-ionone was observed to be the main degradation product, suggesting that bond 9-10 is the most susceptible site to this type of oxidation. As seen earlier, beta-ionone can be oxidized to beta-cyclocitral and dihydroactinidiolide during photosensitized oxidation.

During metachloroperbenzoic acid oxidation, beta-ionone was still the main volatile degradation product, followed by dihydroactinidiolide, beta-cyclocitral and 5,6-epoxy beta-ionone. Perbenzoic acid oxidation is known to oxidize beta-carotene into mainly 5,6-epoxy beta-carotene (Marty and

Berset,1986). If 5,6-epoxy beta-ionone originated from the breakdown of bond 9-10 of 5,6-epoxy beta-carotene, it would be expected that 5,6-epoxy beta-ionone would be the major degradation product. Results obtained rule out such a hypothesis since beta-ionone is the major volatile.

CONCLUSION

Non-volatile degradation products of beta-carotene show polarity very similar to that of beta-carotene and their separation could only be accomplished with normal phase HPLC using a gradient elution system. These degradation products absorb in the same wavelength region as beta-carotene. Furthermore, they may show higher extinction coefficients, which could lead to increased apparent absorption. These compounds were not identified, but their identification should be pursued in future research.

Several compounds have been identified as key volatile oxidative degradation products of beta-carotene. They have been classified with respect to oxidative cleavage of bonds 6-7, 7-8, 8-9 and 9-10 of the beta carotene polyene chain. The main oxidative degradation products were alpha-isophorone, beta-cyclocitral, beta-ionone, 5,6-epoxy beta-ionone and dihydroactinidiolide.

The same volatile compounds were present during all different types of oxidation, however their relative

intensity varied, suggesting that at each oxidation condition, one different bond is more susceptible to cleavage.

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CHAPTER II

KINETICS OF BETA-CAROTENE OXIDATION. PART II BETA-CAROTENE DEGRADATION AND VOLATILE FORMATION IN A MODEL SYSTEM.

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ABSTRACT

The degradation of beta-carotene and concomitant production of oxidative volatile degradation products were investigated in a microcrystalline cellulose model system at temperatures typical of food storage and processing (25, 40, 60 and 80°C). The initial concentration of beta-carotene was typical of its concentration in food systems. Predictions of two previously reported kinetic analyses (a first order model and a free radical recombination approach) and an empirical model (a 1.5 order reaction) were compared with the data. The first order model was the most satisfactory for the loss of beta-carotene over the temperature range investigated. Six volatile products, 2,2,6-trimethyl cyclohexane carboxaldehyde, alpha-isophorone, beta-cyclocitral, beta-ionone, 5,6-epoxy beta-ionone and dihydroactinidiolide, represented 60 to 80% of the total volatile fraction. Measurement of volatile degradation

products, such as dihydroactinidiolide may be a good method for tracking degradation once their production rate is correlated with beta-carotene loss. Such correlation was developed between beta-carotene degradation and production of dihydroactinidiolide and is considered accurate up to losses of 50% of the initial beta-carotene concentration.

INTRODUCTION

Carotenoids are extremely sensitive to oxygen, light and heat. Degradation will lead to loss of color, loss of vitamin A activity and development of off-flavor (Archer and Tannenbaum, 1979). Dehydrated foods are most susceptible to loss of carotenoids because of their tendency to undergo oxidation (Labuza et al., 1970). Since foods are very complex, model systems such as microcrystalline cellulose have been used to investigate the mechanism and kinetics of beta-carotene degradation.

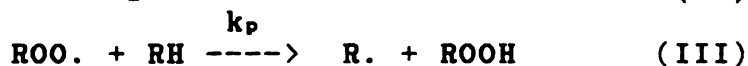
Kinetics of beta-carotene degradation.

Two approaches have been used in the analysis of the kinetics of carotene degradation in model systems: (i) a simplified first order reaction (Chou and Breene, 1972; Arya et al., 1979; Ramakrishnan and Francis, 1979; Stefanovich and Karel, 1982) and (ii) a free radical recombination mechanism involving oxygen attack in several steps (Alekseev et al., 1968; Finkel'stein et al., 1972, 1974; Gagarina et al., 1970; Goldman et al., 1983; Stefanovich and Karel, 1982 and Saguy et al., 1985).

According to Chou and Breene (1972), after an induction period, the rate of decoloration of beta-carotene followed

first order kinetics. First order kinetics are expected if beta-carotene and oxygen are the only reactants present and if a large excess of oxygen is present (Ramakrishnan and Francis, 1979). First order rate constants at different temperatures are summarized in Table 1. There is much variability among measured rate constants. Ramakrishnan and Francis (1979) and Stefanovich and Karel (1982) reported higher rates of reaction as well as activation energies than those observed by other investigators.

In the second approach, the oxidation of beta-carotene was considered an unbranched chain process with a non-stationary or acceleration period associated with the increase in concentration of free radicals, and a period in which beta-carotene oxidation takes place at a constant rate (Finkel'stein et al., 1972). During the oxidation of beta-carotene, the intermediate products formed are also consumed with the same reactivity by chain reactions (Gagarina et al., 1970). The degradation of beta-carotene is then given:



The rate of consumption of beta-carotene in the chain process is, using the same terminology as Stefanovich and Karel (1982):

Table 1. First order rate constants* and activation energies for beta-carotene degradation in model systems.

References	Experimental Conditions		Rate Constant	Activation Energy Ea $\frac{\text{kcal}}{\text{mol}}$
		T°C	k (h ⁻¹)	
El-Tinay and Chichester, 1970	.1 mg/ml beta-carotene toluene	60	3.93×10^{-2}	10.2
		70	6.12×10^{-2}	
		80	10.54×10^{-2}	
		90	14.03×10^{-2}	
Chou and Breese, 1972	0.5% beta-carotene dry Avicel	5	1.2×10^{-3}	9.7
		20	4.4×10^{-3}	
		35	8.8×10^{-3}	
Baloch et al., 1977	? beta-carotene 31% RH microc. cellulose	37	17×10^{-3}	10.3
		44	23.9×10^{-3}	
		50	36.6×10^{-3}	
Ramakrishnan and: Francis, 1979	? beta-carotene dry Avicel			
		25	3.96×10^1	
Arya et al., 1979	1.7 mg/g beta-carotene 33% RH microc. cellulose			
		16-32	$(3.4 \times 10^{-3})^*$	
Stefanovich and Karel, 1982	.14/.57/.89 mg/g beta-carotene dry Avicel	60	0.22/0.23/0.21	23.9/28.9/25.6
		70	0.71/0.85/0.83	
		80	1.43/1.73/1.54	

* Values in parentheses are calculated first order values from literature data.

$$-\frac{dC}{dt} = a C [b_0 C + b(C_0 - C)]^{1/2} \quad (1)$$

$$a = \frac{k_p}{k_t^{1/2}} \quad (2)$$

where: b_0 is the initiation rate constant of unoxidized beta-carotene; b is the initiation rate constant of the products; a is a constant derived from equation (2); C_0 is the initial beta-carotene concentration; C is the concentration of beta-carotene; k_p and k_t are the rate constants of reaction (III) and (IV). The integration of equation (1), and use of the dimensionless variables suggested by Gagarina et al. (1970), yields:

$$\ln \frac{(1 + (1 - C/C_0)^{1/2})}{(1 - (1 - C/C_0)^{1/2})} = a (b - b_0)^{1/2} C_0^{1/2} t \quad (3)$$

Stefanovich and Karel (1982) investigated the applicability of this approach, however it did not prove to be entirely satisfactory since an induction period was observed and the kinetic parameters obtained were dependent on the initial concentration. Equation (1) has the rate of beta-carotene loss proportional to the 1.5 power of its concentration. This suggests a simple power law to fit the data with $n=1.5$.

$$-\frac{dC}{dt} = kC^{1.5}$$

Through integration we would have:

$$C_0^{1/2} - C^{1/2} = kt \quad (4)$$

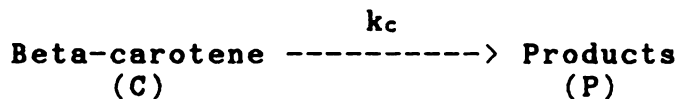
This model would be a much simpler approach to that of Gagarina et al. (1970).

In every one of these investigations on the kinetics of beta-carotene degradation, the loss of beta-carotene was monitored by direct spectral measurement. We showed in Chapter I of this study that simple measurement of absorbance is not a valid method for measuring beta-carotene degradation. Also, the rates of product formation were not related to the rate of beta-carotene disappearance.

Kinetics of volatile formation.

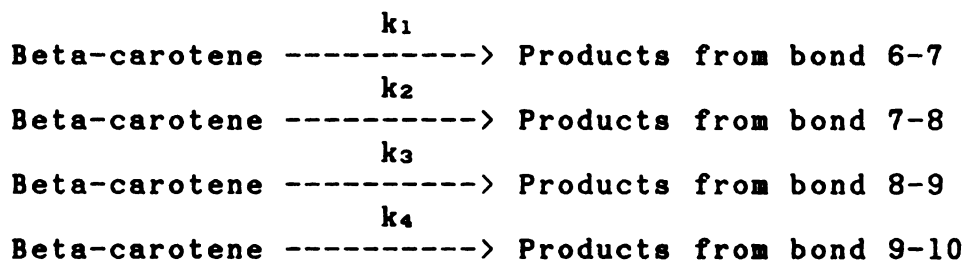
We categorized volatile degradation products from beta-carotene according to the bond cleavage in the polyene backbone. Oxidative cleavage of beta-carotene occurred at bonds 6-7, 7-8, 8-9 and 9-10, leading to the formation of C₉, C₁₀, C₁₁ and C₁₃ compounds. Among compounds identified, alpha-isophorone, beta-cyclocitral, beta-ionone, 5,6-epoxy beta-ionone and dihydroactinidiolide gave significant contribution to the total volatile fraction.

If a first order rate equation satisfactorily fits the data for beta-carotene degradation at temperatures of 25, 40, 60 and 80°C in excess oxygen:



$$-\frac{dC}{dt} = k_c(C) = -r_c \quad (5)$$

During oxidation of beta-carotene, bonds 6-7, 7-8, 8-9 and 9-10 were shown to be the main sites of oxygen attack. Since oxygen attack to these bonds cannot happen concomitantly in the same molecule, beta-carotene is decomposing mainly by four different pathways. There are 4 competing elementary first order reactions:



Compounds contributing to products from each bond are listed in Table 2. There are other degradation products which do not have oxygen in them and thought to be formed by rearrangement reactions. These products are aromatic compounds and may not contribute to hay like off-flavors.

The rates of formation of compounds from the 4 parallel reactions are:

$$-r_c = -\frac{dC}{dt} = k_c C = k_1 C + k_2 C + k_3 C + k_4 C = \frac{dP_i}{dt} \quad (6)$$

$$r_1 = \frac{dP_1}{dt} = k_1 C = -\frac{dC}{dt} \quad (7)$$

$$r_2 = \frac{dP_2}{dt} = k_2 C = -\frac{dC}{dt} \quad (8)$$

$$r_3 = \frac{dP_3}{dt} = k_3 C = -\frac{dC}{dt} \quad (9)$$

Table 2. Volatile compounds formed from cleavage of beta-carotene at bonds 6-7, 7-8, 8-9 and 9-10 during autoxidation of beta-carotene in Avicel model system at 25, 40, 60 and 80°C under excess oxygen.

Bond	Compounds
6-7	2,2,6-trimethyl cyclohexanone 3,3,5-trimethyl cyclohexanone alpha-isophorone beta-isophorone
7-8	2,2,6-trimethyl cyclohexane 1 carboxaldehyde beta-cyclocitral
8-9	dihydroactinidiolide
9-10	alpha-ionone beta-ionone 5,6-epoxy beta-ionone

$$r_4 = \frac{dP_4}{dt} = k_4 C = - \frac{dC}{dt} \quad (10)$$

By using all 5 differential equations:

$$- \ln \frac{C}{C_0} = (k_1 + k_2 + k_3 + k_4)t = k_c t \quad (11)$$

By dividing equation (7) by equation (9) we obtain:

$$\frac{r_1}{r_3} = \frac{dP_1}{dP_3} = \frac{k_1}{k_3}$$

which gives when integrated:

$$\frac{P_1 - P_{10}}{P_3 - P_{30}} = \frac{k_1}{k_3} \quad (12)$$

According to Levenspiel (1972) the plot of P_1 vs P_3 should be linear for parallel first order equations. The slope of P_1 vs P_3 will give the ratios k_1/k_3 . Knowing values for k_1/k_3 , k_2/k_3 and k_4/k_3 as well as that:

$(k_1 + k_2 + k_3 + k_4) = k_c$, we can estimate values for the rate constants of compound formation from bonds 6-7, 7-8, 8-9 and 9-10, respectively: k_1 , k_2 , k_3 and k_4 .

From equation (4):

$$-\frac{dC}{dt} = k_c t$$

$$\ln \frac{C}{C_0} = - k_c t \quad (13)$$

$$\text{then, } C = C_0 \exp (-k_c t) \quad (14)$$

and from equations (7-10):

$$\frac{dP}{dt} = k_i C \quad (15)$$

by substituting (14) in (15):

$$\frac{dP_i}{dt} = k_i C_o \exp(-k_c t) \quad (16)$$

and after integration:

$$P_i = - \frac{k_i C_o}{k_c} \exp(-k_c t) + C_1 \quad (17)$$

where k_c is the rate constant for beta-carotene loss and k_i is the rate for formation of compounds from cleavage of bond i . When $t = 0$, $\exp(-k_c t) = 1$ and $P = 0$, the constant C_1 will be: $C_1 = \frac{k_i C_o}{k_c}$, leading to the expression:

$$P_i = \frac{k_i C_o}{k_c} (1 - \exp(-k_c t)) \quad (18)$$

The rate constants follow the Arrhenius equation:

$$k_c = k_{c0} \exp(-E_{ac}/RT) \quad (19)$$

$$\text{and} \quad k_i = k_{i0} \exp(-E_{ai}/RT) \quad (20)$$

where E_{ac} and E_{ai} and k_{c0} and k_{i0} , are the activation energy and preexponential (frequency) factor for beta-carotene degradation and volatile formation respectively.

Substituting k_c and k_i in equation (18):

$$P_i = \frac{k_{i0}}{k_{c0}} \exp\left(-\frac{(E_{ai} - E_{ac})}{RT}\right) C_o [1 - \exp\left(-\frac{k_{c0} \exp(-\frac{E_{ac}}{RT})}{t}\right)] \quad (21)$$

The objective of this study was to (i) determine the effect of temperature on the degradation of beta-carotene and concomitant formation of volatile degradation products; and (ii) to relate beta-carotene loss to volatile formation

in order to predict beta-carotene loss by the presence of volatiles and vice-versa.

EXPERIMENTAL PROCEDURES

Model system preparation.

Synthetic all-trans-beta-carotene (Sigma Chemicals, St. Louis, MO) was purified by column chromatography using alumina as adsorbent. The purity was tested by HPLC. Microcrystalline cellulose-Avicel type RC-591 (FMC Corporation, Philadelphia, PA) was used as a solid support. It was dried in an oven at 80°C for 24 hours. A petroleum ether solution containing beta-carotene was mixed with Avicel (1mg/g) until the color distribution was visually homogeneous. Residual petroleum ether was removed under vacuum. All steps were carried out under yellow light (Gold fluorescent bulbs F40G06PK, General Electric, Cleveland, OH).

Effect of temperature.

Forty grams of the model system were placed in a round bottom flask, covered with aluminum foil, and immersed in a paraffin bath at temperatures of 25, 40, 60 and 80°C. An oxygen tank was attached to the system, furnishing oxygen at a flow of 10 ml/min. As a control, oxygen absorbers (Sakamaki, 1986) and a nitrogen flow of 30 ml/min were used to ensure an oxygen free atmosphere at 80°C. Oxy-trap

(Alltech Associates, Deerfield, IL) was used to remove residual oxygen from the nitrogen. Periodically, samples were withdrawn from the reaction vessel and analyzed for beta-carotene content and volatile degradation products. Results represent duplicate analyses of five different samples.

Analysis of beta-carotene.

Beta-carotene was extracted and analysed by HPLC as described in Chapter I.

Volatile degradation products.

The volatiles formed by the oxidation of beta-carotene were collected by a Tenax GC trap system, separated and quantitated by GC and identified by GC/MS as described in Chapter I.

RESULTS AND DISCUSSION

Stability of beta-carotene.

Figure 1 shows the relationship between beta-carotene loss and reaction time at 25, 40, 60 and 80°C under oxygen and at 80°C under nitrogen (control). In the absence of oxygen, there was no change in the beta-carotene content with time, indicating that beta-carotene is stable at 80°C (for up to 138 hours) under an inert atmosphere. These results are different from those of previous workers. Baloch

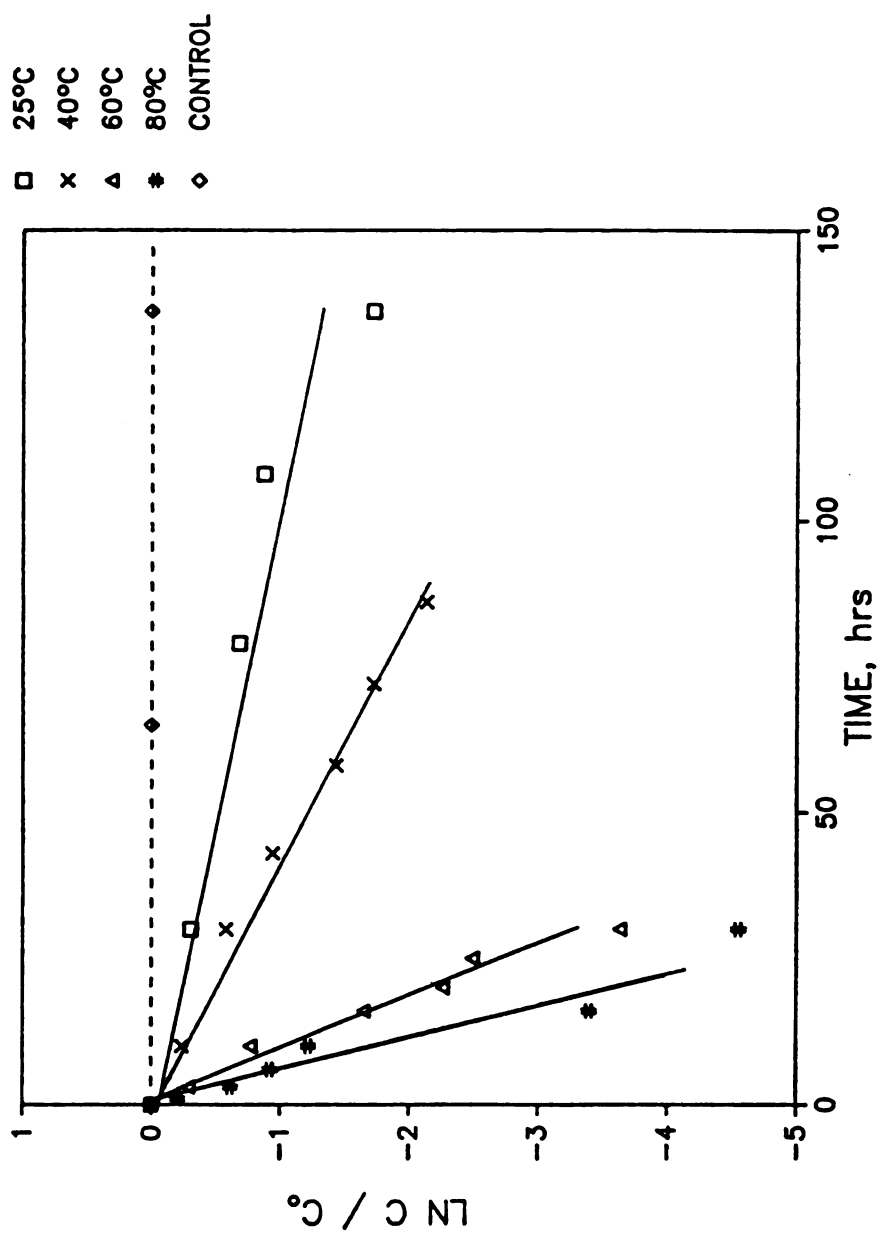


Figure 1. Autoxidation of beta-carotene in Avicel model system under excess oxygen at 25, 40, 60 and 80°C.

et al. (1977) and Goldman et al. (1983) observed loss of beta-carotene under nitrogen, however, this loss could be due to oxygen adsorbed or entrapped by the system. The loss of beta-carotene at all temperatures investigated could be described by a first order reaction for the loss of beta-carotene, as it gives very good correlation for linear regression ($r^2 > 0.9809$ for all replicate trials).

First order analysis.

Calculated first order rate constants for each temperature, are indicated in Table 3. The rate constants for the degradation of beta-carotene in model systems as summarized in Table 1 are significantly lower than experimental values (Table 3). However, since in all those investigations beta-carotene content was measured by its absorbance at 450 nm, it is likely that they were also including absorbance due to beta-carotene degradation products along with that of beta-carotene content. Their reported values would be higher than the actual beta-carotene concentration, giving lower rate constants.

Trace metals present in the model system support could act as metallic catalysts, thus affecting rate constants. The support used in this investigation contained iron (1.4 ± 0.36 ug/g) and copper (0.27 ± 0.06 ug/g) measured by atomic absorption analysis. The presence of these metals could increase the rate of autoxidation. However their effect cannot be measured as there is no reference to the metal

Table 3. First order, free radical recombination and empirical approach rate constants and activation energies for beta-carotene loss during autoxidation at 25, 40, 60 and 80°C in Avicel model system under excess oxygen.

		Rate Constant $k(\text{hr}^{-1})$		
Autoxidation at T°C		First order	Free radical recombination	Empirical approach
		(Eq. 5)	(Eq. 22)	(Eq. 4)
25	k	0.0086	0.0198	0.0344
		(0.0006)	(0.001)	(0.0003)
	r^2	.9911	.9411	.9701
40	k	0.0191	0.0499	0.0772
		(0.0046)	(0.0066)	(0.0041)
	r^2	.9809	.9013	.9897
60	k	0.0997	0.2041	0.2748
		(0.0240)	(0.0286)	(0.0051)
	r^2	.9999	.9428	.9640
80	k	0.2053	0.5238	0.2985
		(0.0058)	(0.0082)	(0.0034)
	r^2	.9999	.9353	.8736
Ea Kcal/ mol		12.64	12.65	8.74

¹ Values represent means of five replications which were analyzed in duplicate.

² Standard deviations are shown in parentheses.

content in the supports used in other investigations. Further research is needed in order to elucidate the role of metal catalysts on the autoxidation of beta-carotene in model systems.

The activation energy (Figure 2) obtained in the model system (25-80°C) was 12.64 ± 0.25 kcal/mol. This value is in the range of values reported by El-Tinay and Chichester (1969), Chou and Breene (1972) and Baloch et al. (1977) (Table 1). However, the activation energy reported by Finkel'stein et al. (1973) and Stefanovich and Karel (1982) was much higher. They suggested that there could be two regions of temperature dependence due to phase transition in the beta-carotene but did not indicate to which region their constants referred.

Free radical recombination analysis.

The experimental data were also analyzed by the free radical recombination approach. Using the same terminology of Saguy et al. (1985), all the constants in the right-hand side of equation (3) were denoted as " $\sqrt{}$ " effective rate constant ($\sqrt{} = a (b-b_0)^{1/2} C_0^{1/2}$), leading to:

$$\ln \frac{(1 + (1-C/C_0)^{1/2})}{(1 - (1-C/C_0)^{1/2})} = \sqrt{} t \quad (22)$$

A plot of the left hand side of Eq. 19 vs t should give a straight line with slope $\sqrt{}$ (Figure 3). The effective rate constants are listed in Table 3. They are much higher than the first order rate constants, however, they lead to

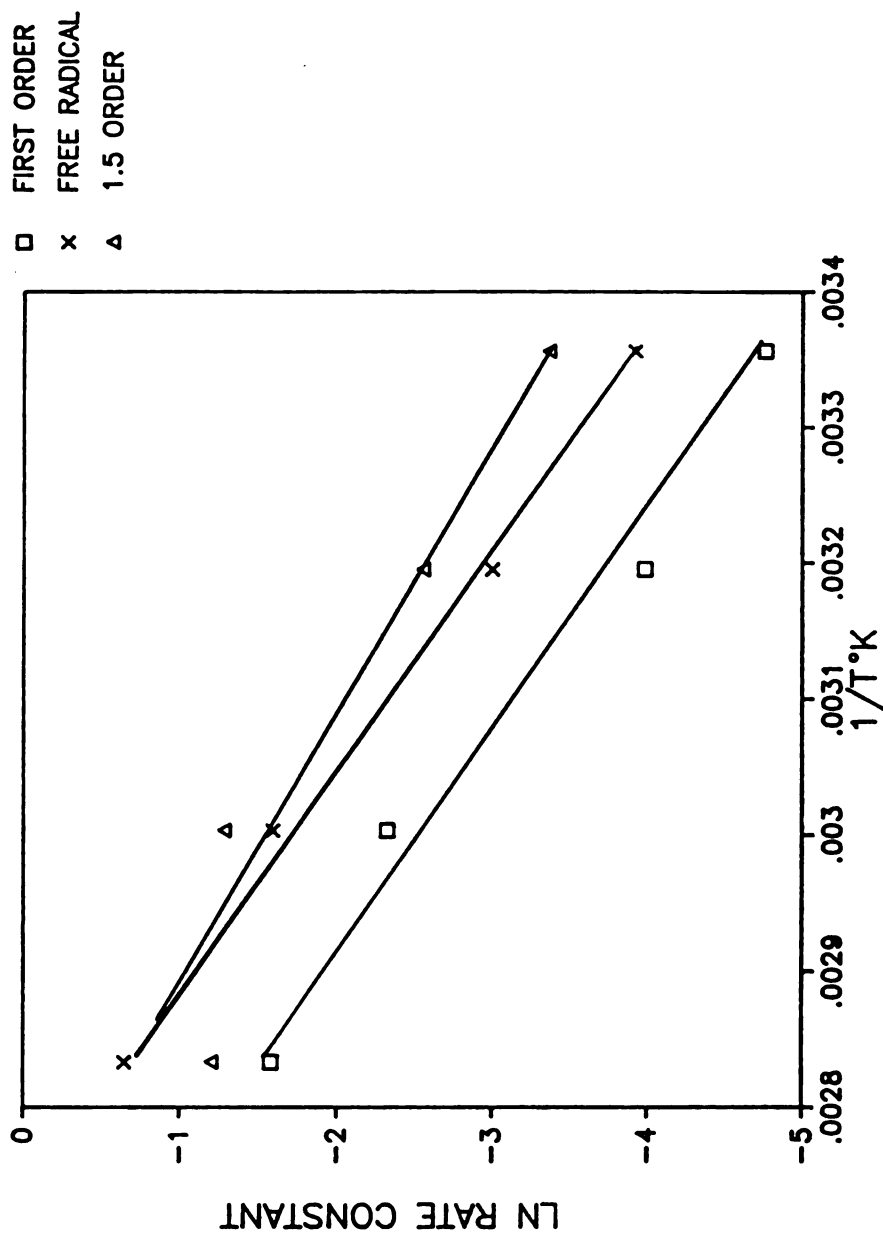


Figure 2. Activation energy for the autoxidation of beta-carotene in Avicel model system under excess oxygen at 25, 40, 60 and 80°C by the first order, free radical recombination and empirical approaches.

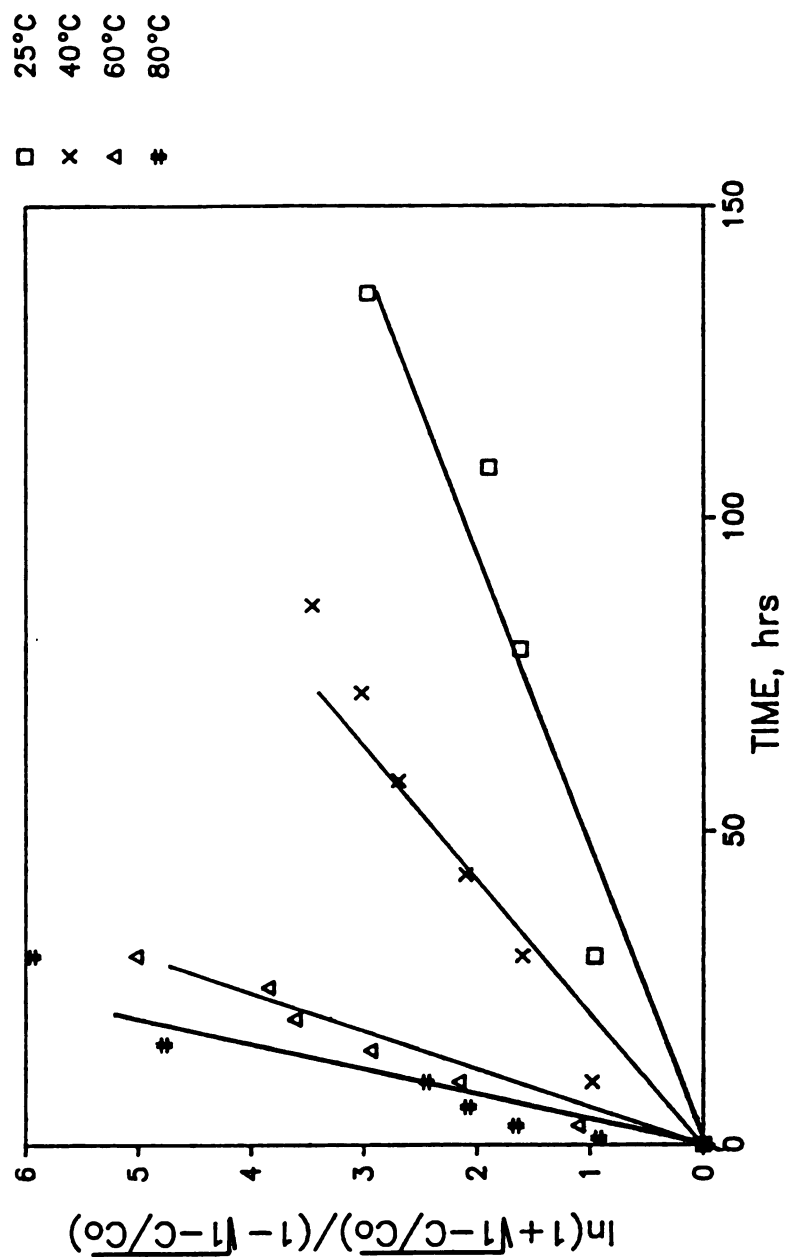


Figure 3. Free radical recombination kinetics of beta-carotene autoxidation in Avicel model system under excess oxygen at 25, 40, 60 and 80°C.

the same activation energy ($12.65 + 1.39$ kcal/mol) as the one for the first order approach. The fit of the free radical recombination model to the data shows a consistent bias. The data in Figure 3 should be in a straight line going through the origin. In all cases, the model underpredicts and then overpredicts the data as time increases.

Empirical model analysis.

Figure 4 shows the plot of $C_0^{1/2} - C^{1/2}$ vs t (Equation 4) for beta-carotene. The activation energy was calculated to be 8.74 Kcal per mole. The empirical model does not show the bias exhibited by the free radical recombination. As one compares the correlation for the linear regression for the loss of beta-carotene by the different approaches tested, the data fit best the first order approach, followed by the empirical approach proposed, and last by the free radical recombination mechanism proposed by Gagarina et al. (1970).

Volatile degradation products.

Figures 5 illustrate the change in the volatile oxidative degradation products profile with time at temperatures of 25, 40, 60 and 80°C. At all temperatures, six volatile degradation products accounted for 60 to 80% of the total volatile fraction: 2,2,6-trimethyl cyclohexane carboxaldehyde, alpha-isophorone, beta-cyclocitral, beta-ionone, 5,6-epoxy beta-ionone and dihydroactinidiolide.

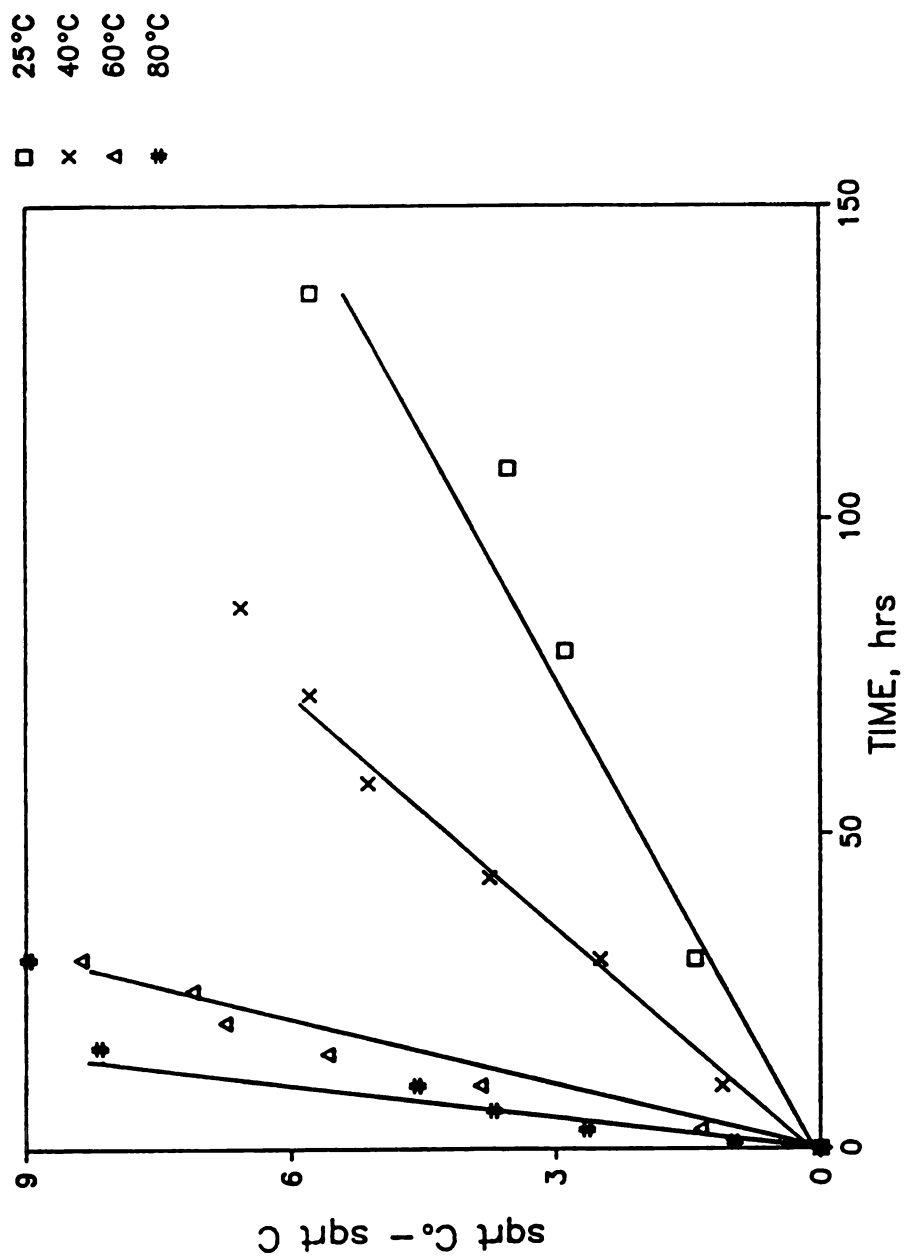
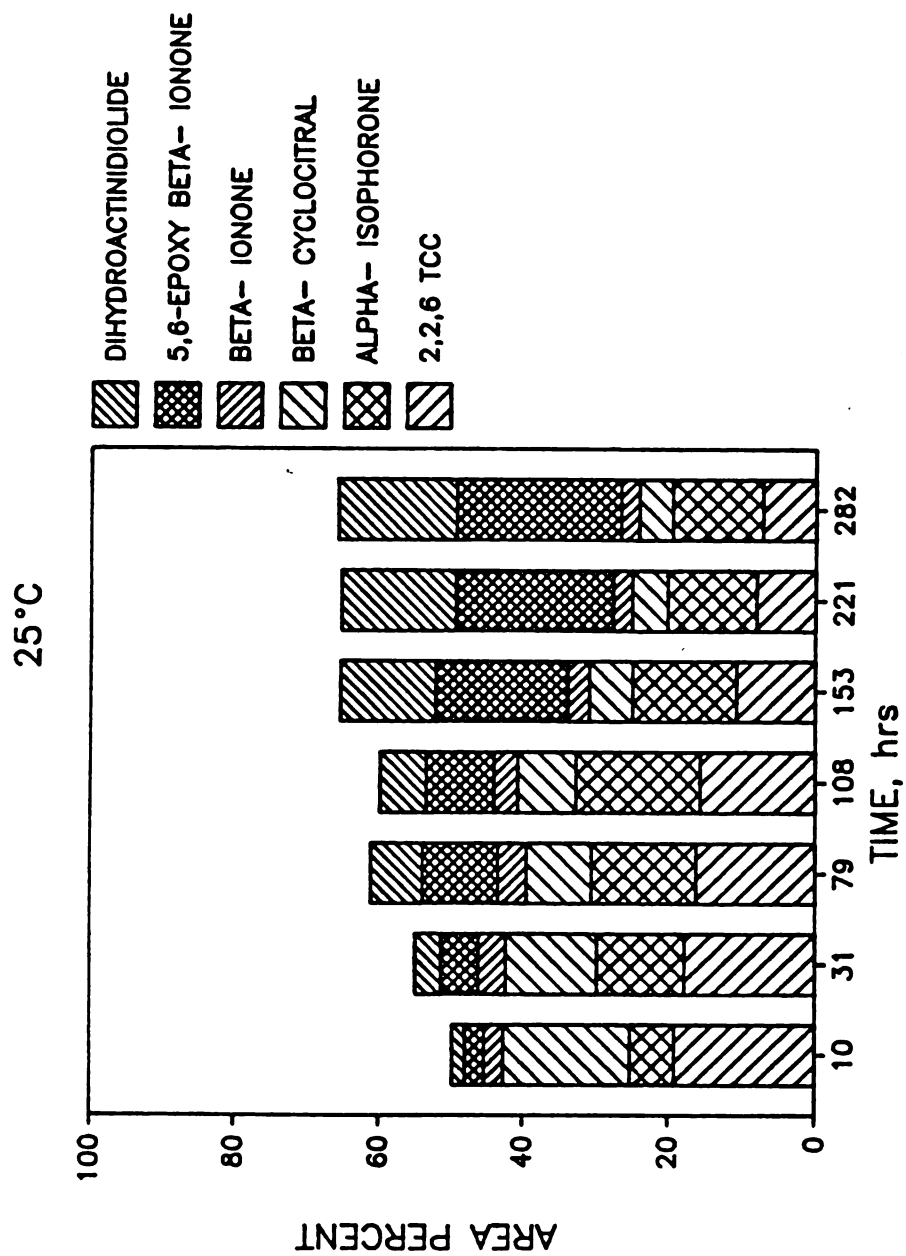
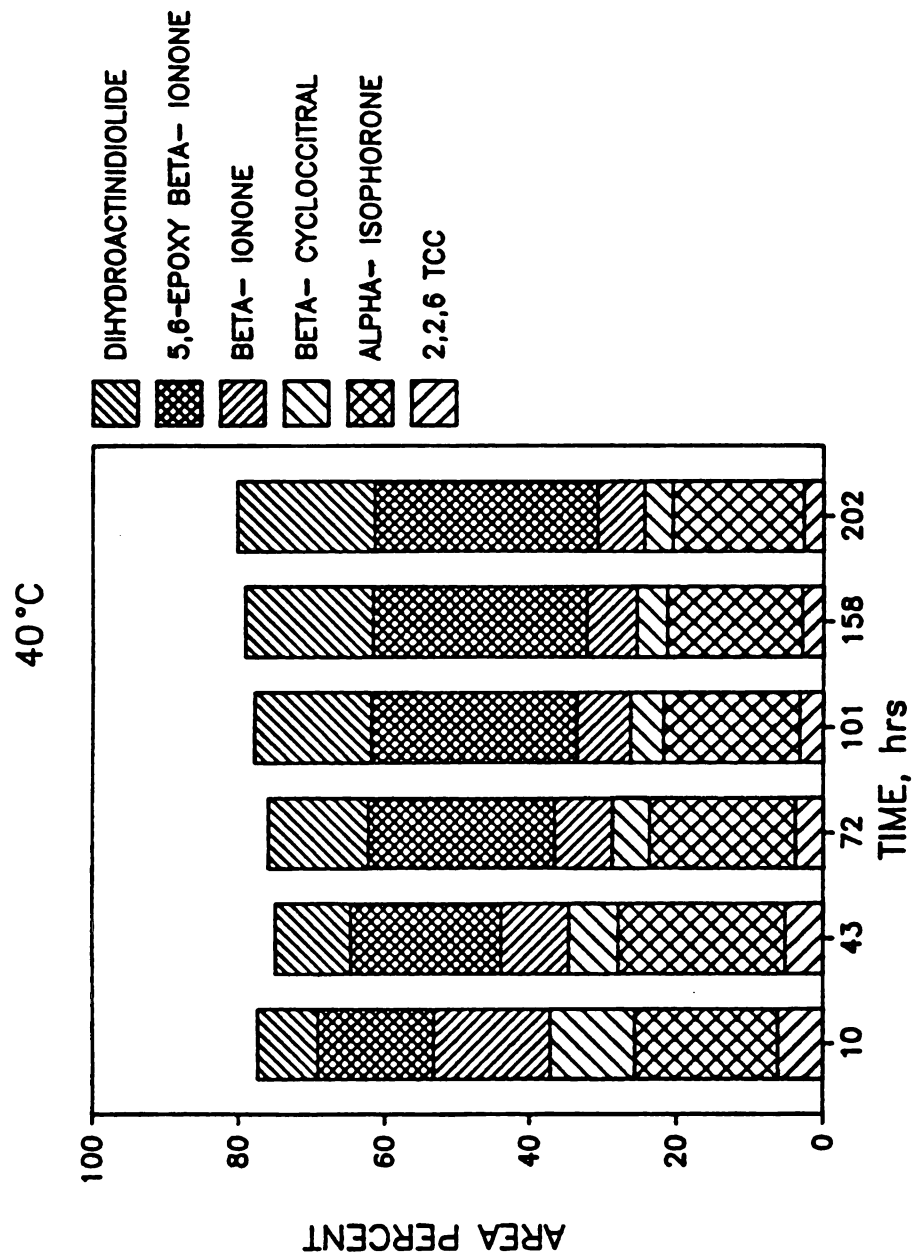
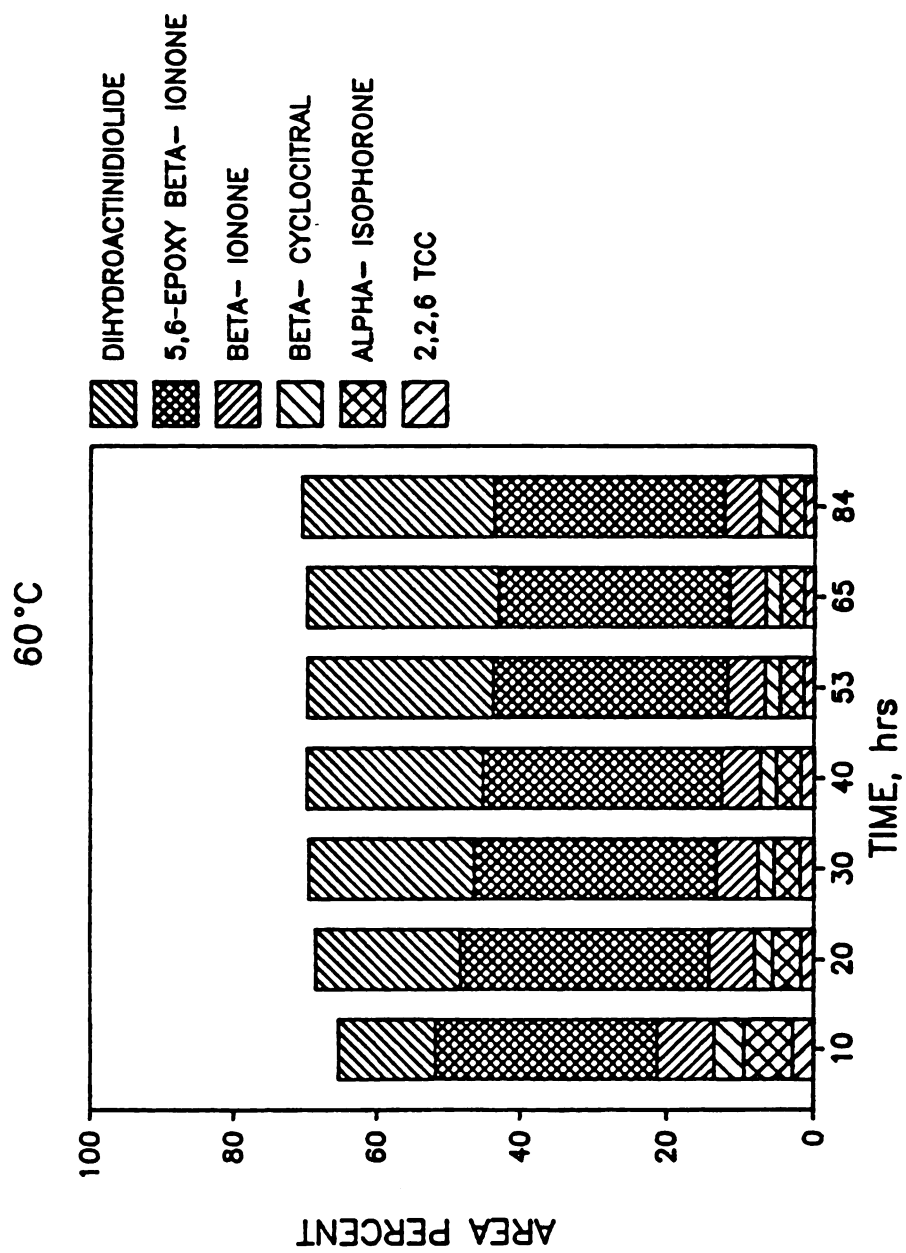


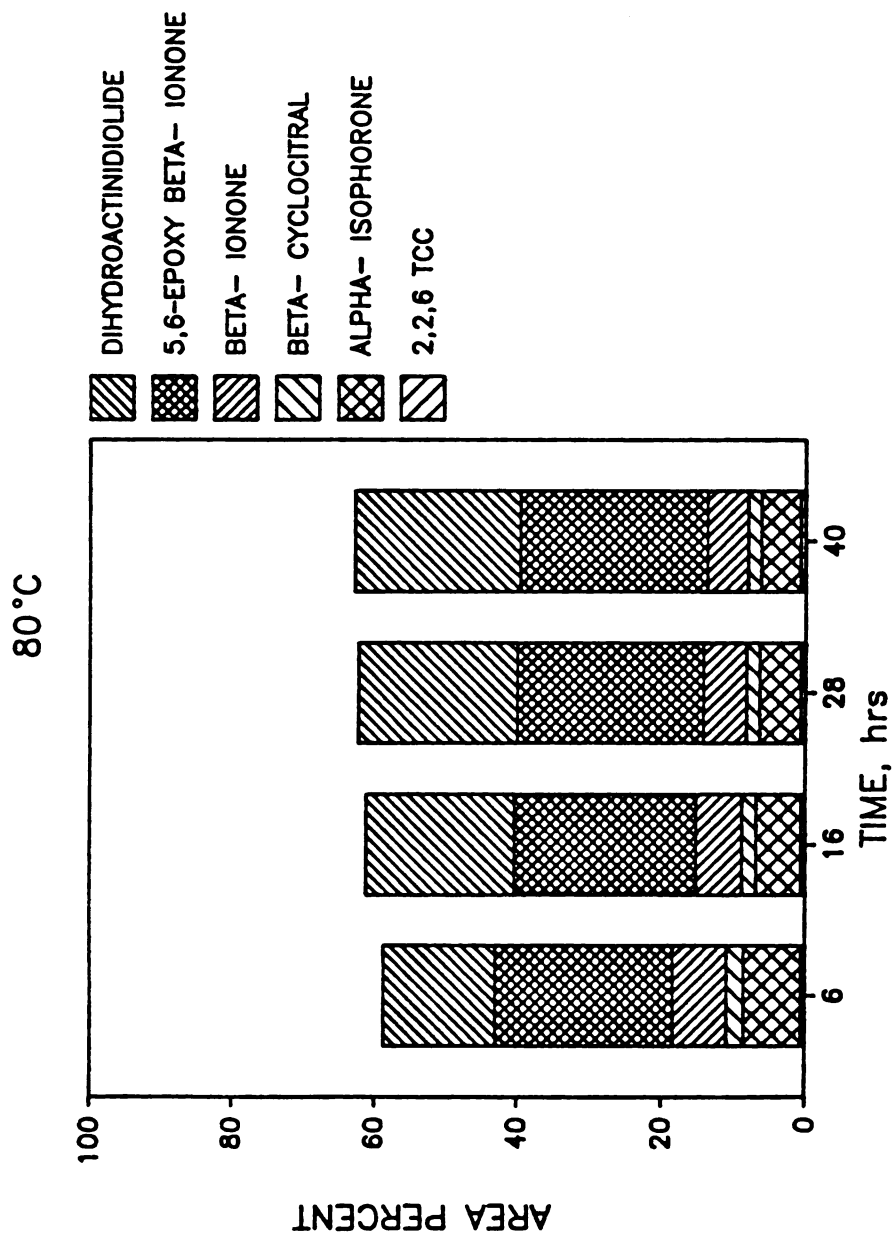
Figure 4. Kinetics of beta-carotene autoxidation in Avicel model system under excess oxygen at 25, 40, 60 and 80°C by the empirical approach.

Figure 5. Change in the volatile degradation products profile with time during autoxidation of beta-carotene in Avicel model system under excess oxygen at 25, 40, 60, and 80°C.









Several other compounds were present but their relative individual contribution was not significant.

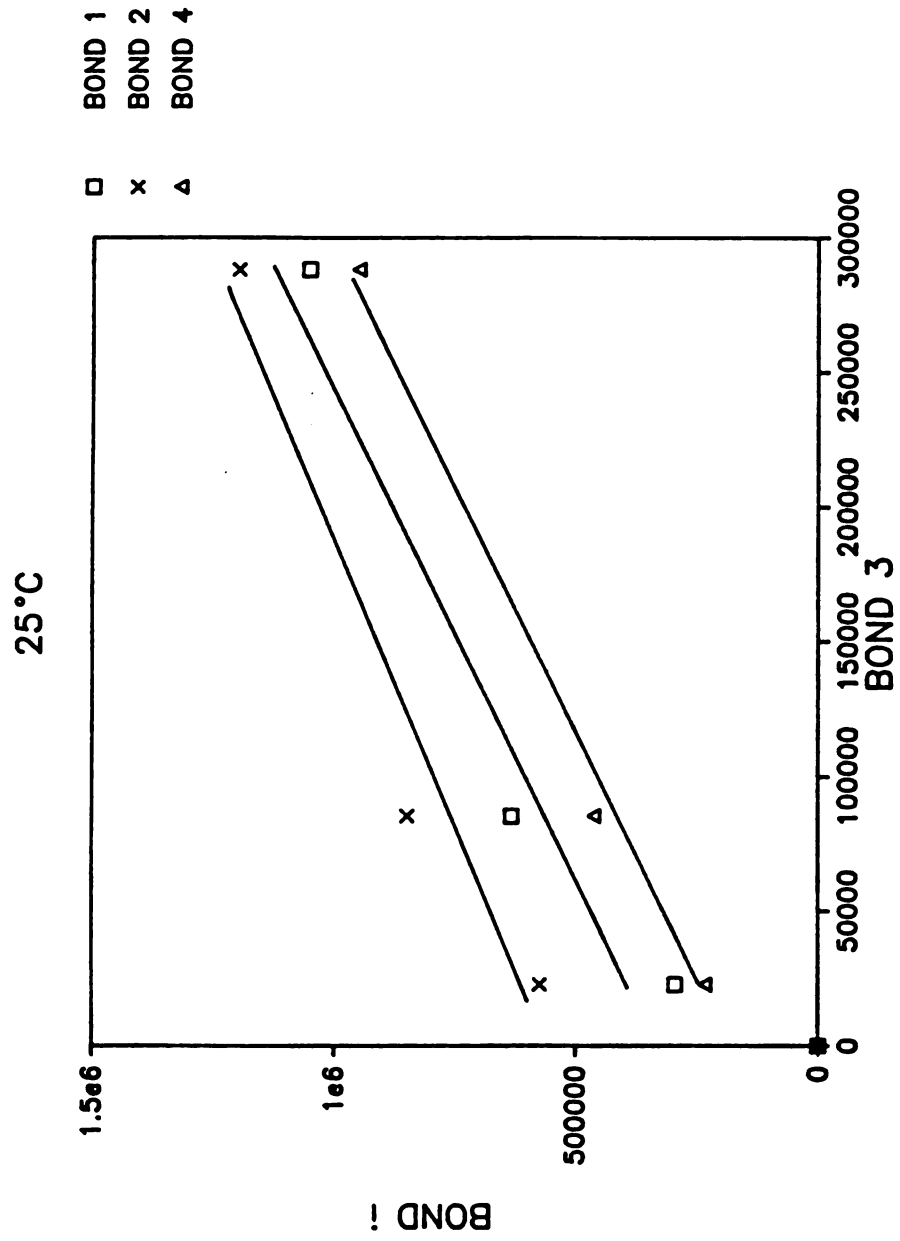
At all investigated temperatures, there was a significant continuous increase in the percentual contribution of dihydroactinidiolide and 5,6-epoxy beta-ionone to the total volatile fraction with oxidation time. However, a decrease was observed for 2,2,6-trimethyl cyclohexane carboxaldehyde (2,2,6 TCC), beta-cyclocitral and beta-ionone. In the case of alpha-isophorone, its contribution decreased for the temperatures of 60 and 80°C. Since beta-ionone, dihydroactinidiolide and 5,6-epoxy beta-ionone have been described as having hay-like flavor, prediction of their concentration is desirable.

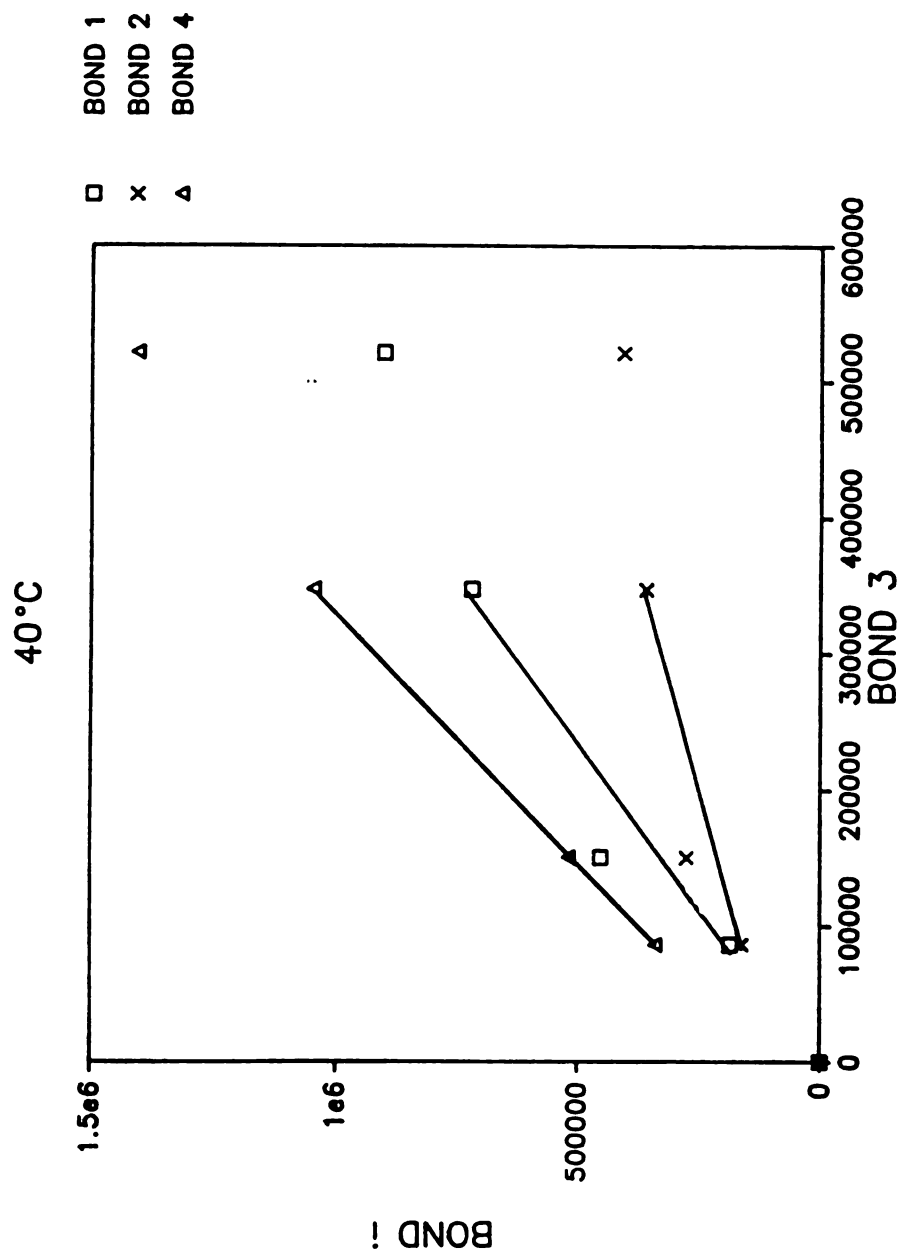
Mechanism for beta-carotene degradation into volatile degradation products.

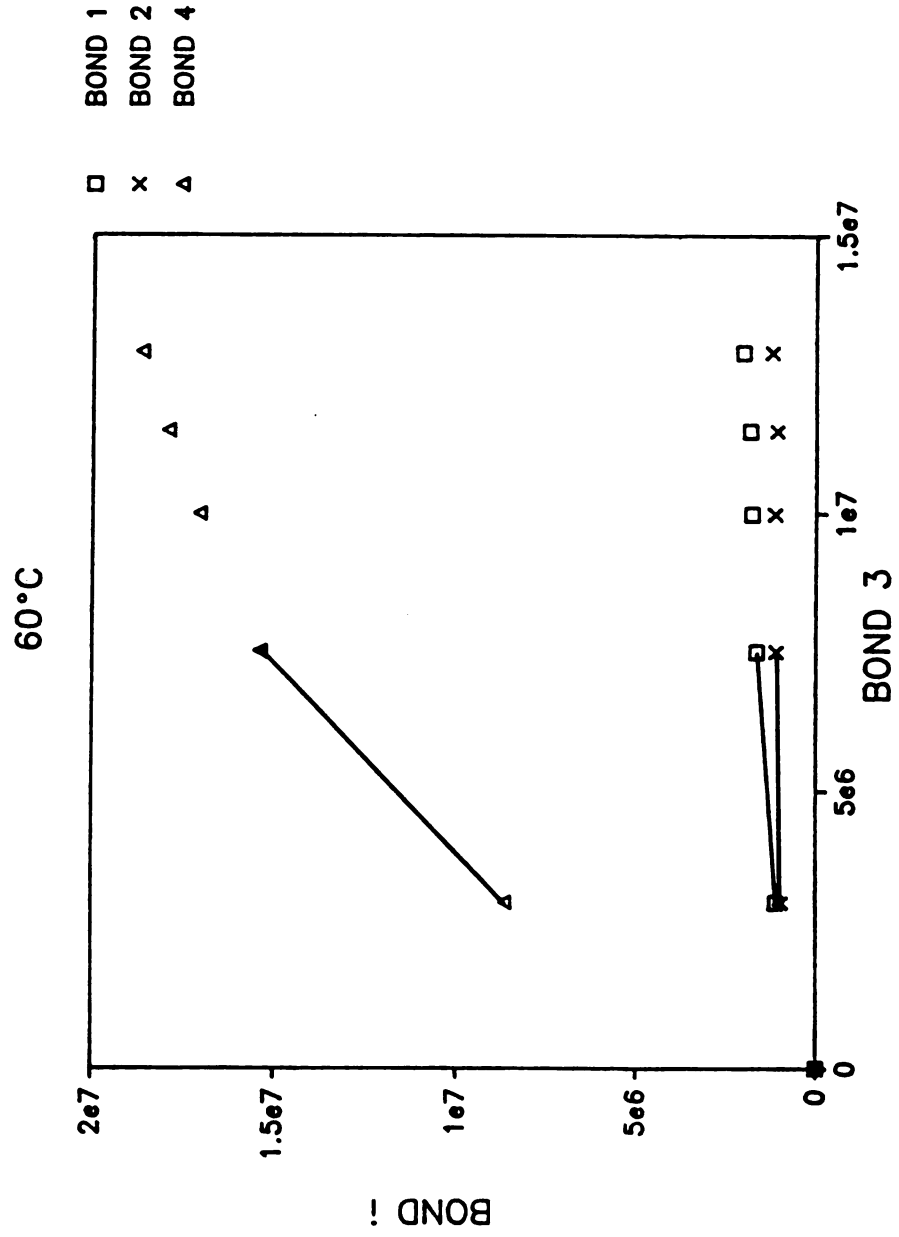
Since beta-carotene autoxidation fits first order kinetics and the plots of P_1 vs P_3 are linear (Figure 6), the model proposed by Levenspiel (1972) can be applied. P_3 was chosen as a reference as it behaves in a similar way for all temperatures and it is represented by only one compound: dihydroactinidiolide. Furthermore, according to Chapter I of this study, dihydroactinidiolide is not generated from autoxidation of other degradation products.

Calculated rate constants for the formation of volatile degradation products according to Levenspiel's approach, are listed in Table 4. Figure 7 and Table 5 show the temperature

Figure 6. Plot of P_1 versus P_3 during autoxidation of beta-carotene in Avicel model system under excess oxygen at 25, 40, 60 and 80°C.







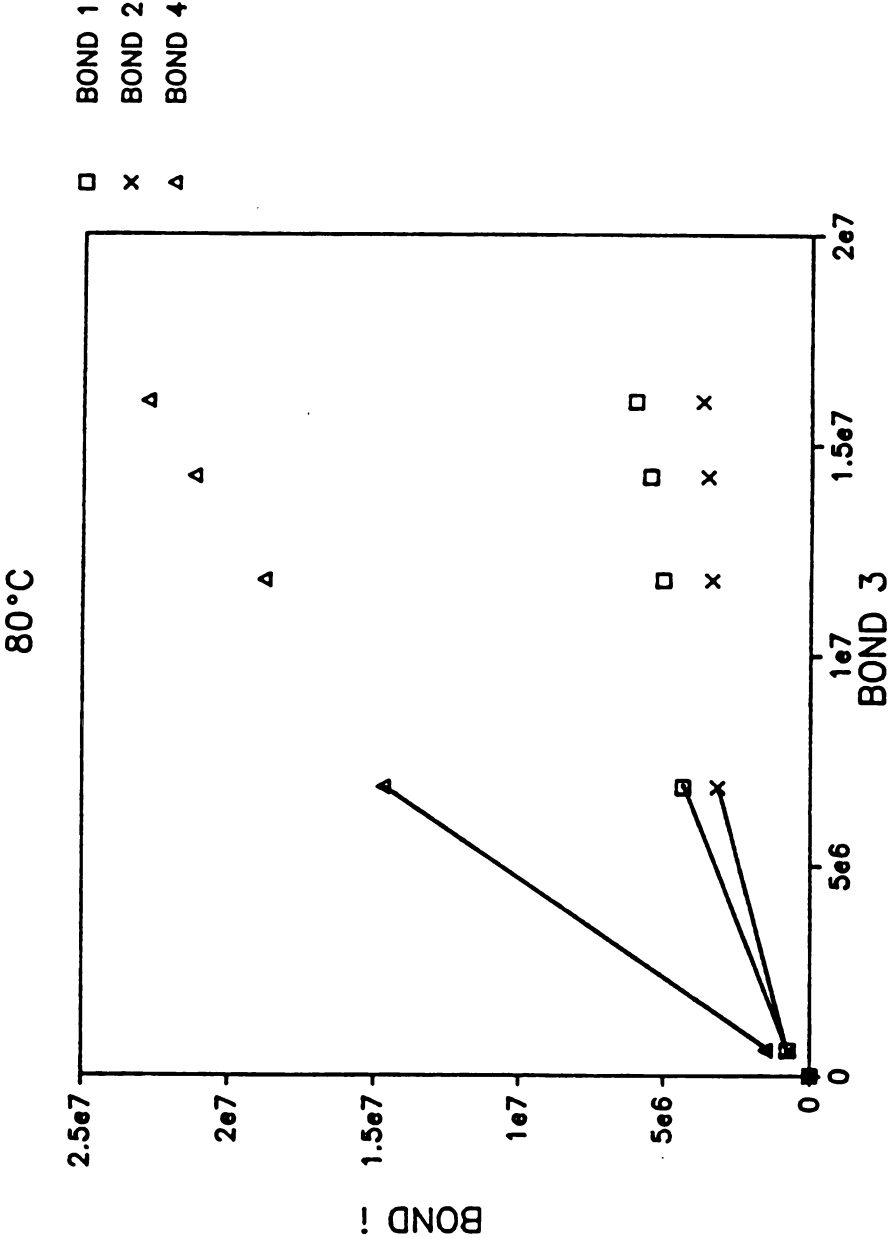


Table 4. First order rate constants* for the formation of breakdown of products from bonds 6-7, 7-8, 8-9 and 9-10 of beta-carotene in Avicel model system at 25, 40, 60 and 80°C under excess oxygen up to 50% loss of beta-carotene.

Bond	Rate Constant ¹ (10 ² hr ⁻¹) at T°C			
	25	40	60	80
6 - 7	0.27 (0.002)	0.53 (0.08)	0.22 (0.04)	2.92 (0.80)
7 - 8	0.23 (0.02)	0.19 (0.02)	0.13 (0.03)	2.14 (0.012)
8 - 9	0.10 (0.0009)	0.32 (0.03)	4.01 (0.20)	4.88 (0.62)
9 - 10	0.27 (0.004)	0.86 (0.06)	5.61 (0.53)	10.60 (1.3)

* Values represent means of five replications analyzed in duplicate.

¹ Standard deviations are shown in parentheses.

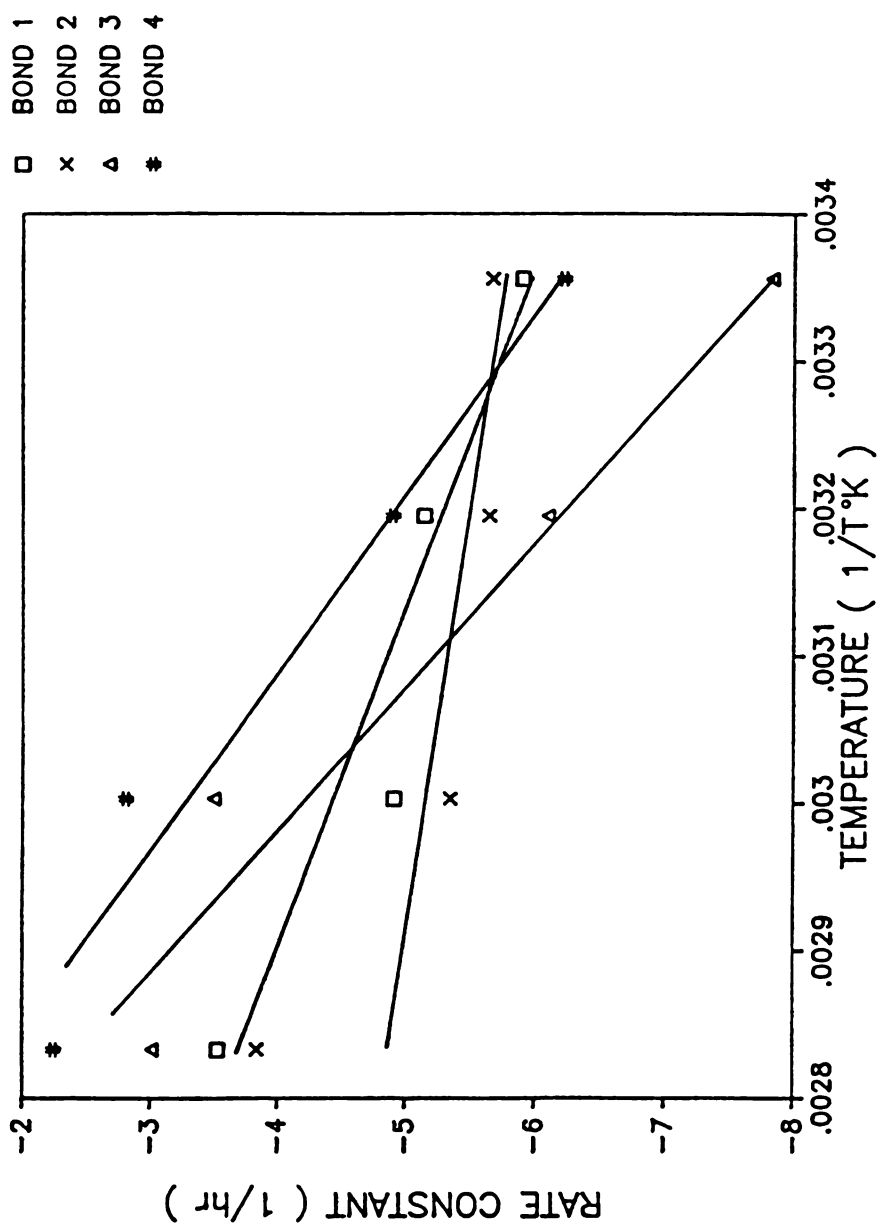


Figure 7. Arrhenius plot for the formation of volatiles from beta-carotene by cleavage at bonds 6-7, 7-8, 8-9 and 9-10 during autoxidation of beta-carotene in Avicel model system under excess oxygen, at 25-80°C.

Table 5. Activation energy* for the formation of volatile products from bonds 6-7, 7-8, 8-9 and 9-10 of beta-carotene and for beta-carotene degradation in Avicel model system at 25, 40, 60 and 80°C under excess oxygen.

Bond	Activation Energy Kcal/mol	Preexponential factor
6-7	6.66 (.9078)	188.7
7-8	6.94 (.7404)	175.9
8-9	19.38 (.9512)	8.37×10^{10}
9-10	14.68 (.9668)	1.75×10^8
beta-carotene degradation	12.64 (.9769)	1.62×10^7

* Correlation for linear regression is shown in parentheses.

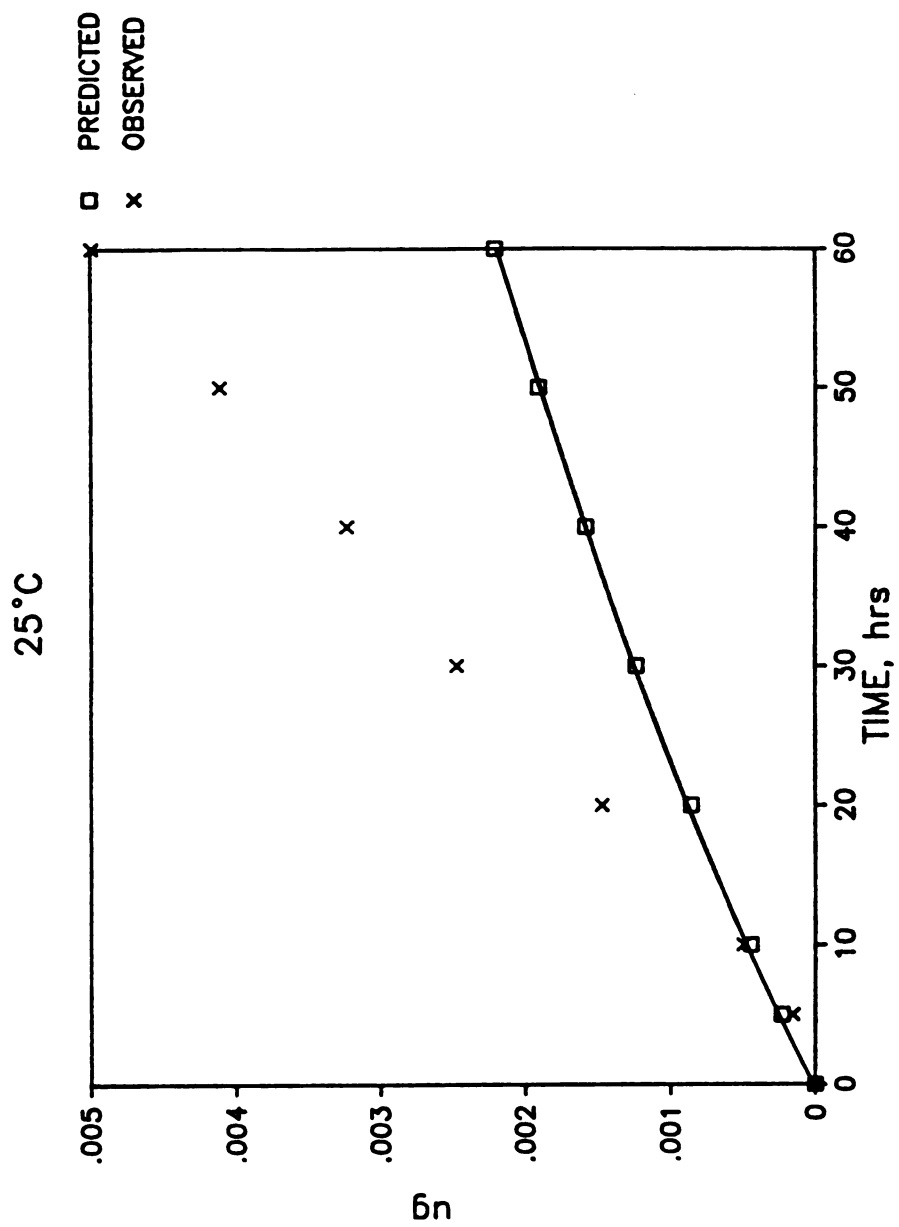
dependency of the rate of formation of volatile degradation products. Using activation energy values and frequency factors in equation (18), we can develop a model to predict volatile formation. According to previous observation, the monitoring of dihydroactinidiolide (as P_3) would be a good reference for monitoring beta-carotene oxidation. The concentration of dihydroactinidiolide during oxidation of beta-carotene can be described by:

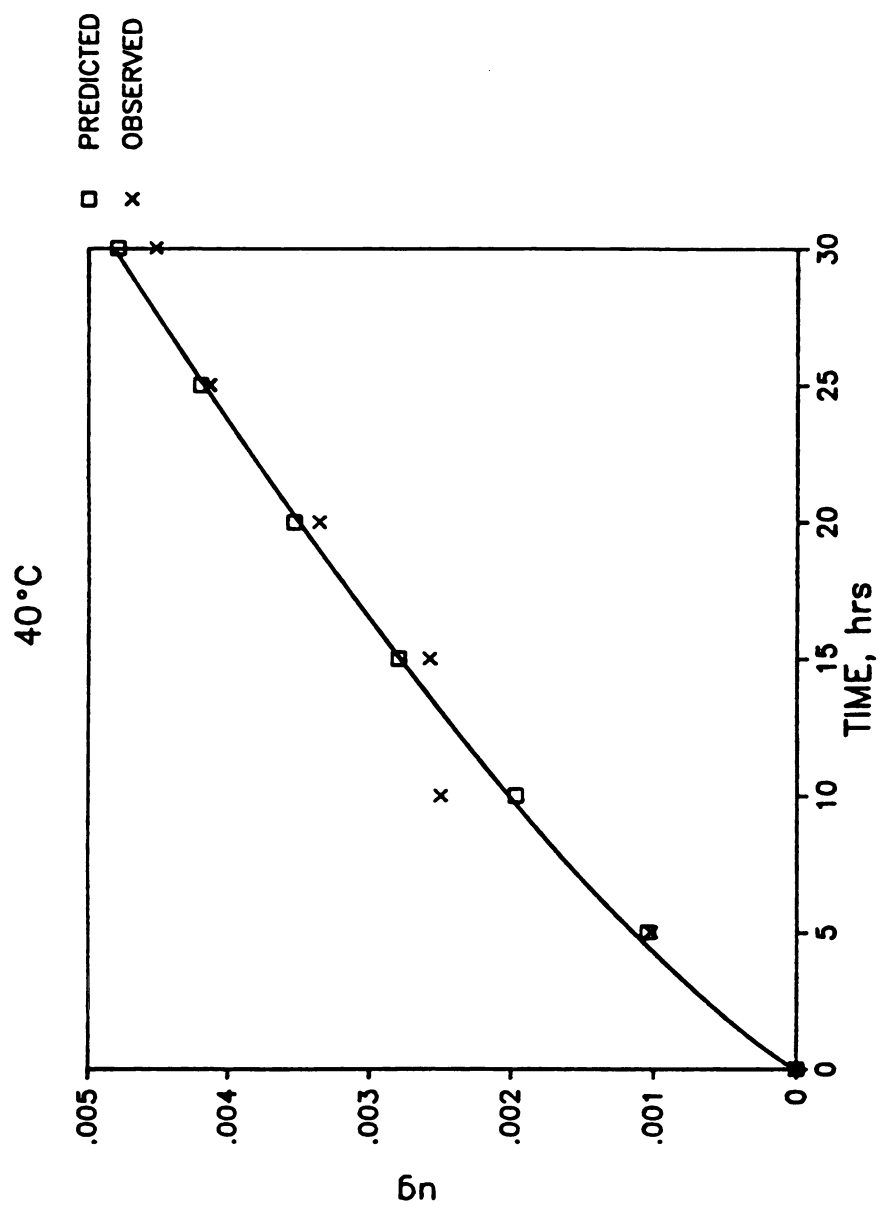
$$P_3 = 5167 C_0 \exp\left(-\frac{6740}{RT}\right) [1 - \exp(-1.62 \times 10^7 \exp\left(-\frac{12640}{RT}\right) t)]$$

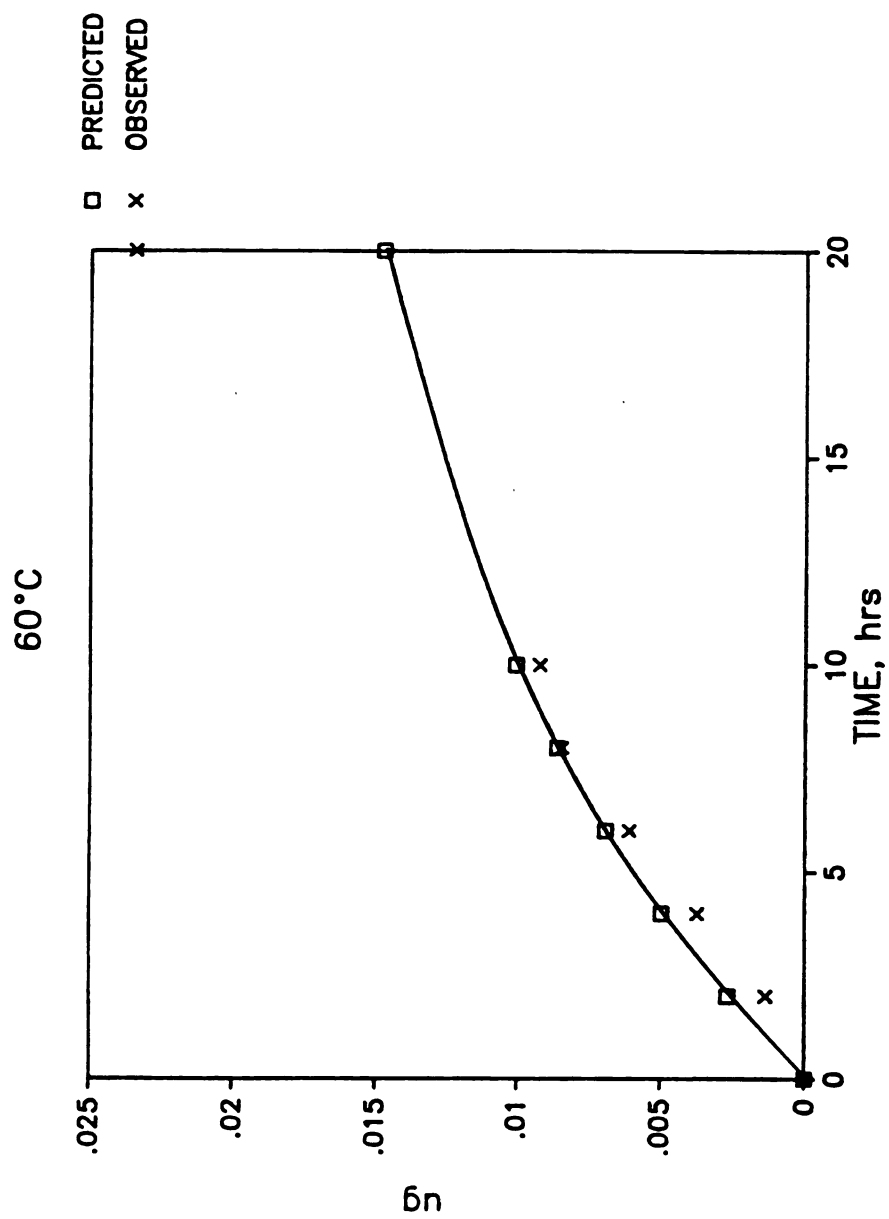
(23)

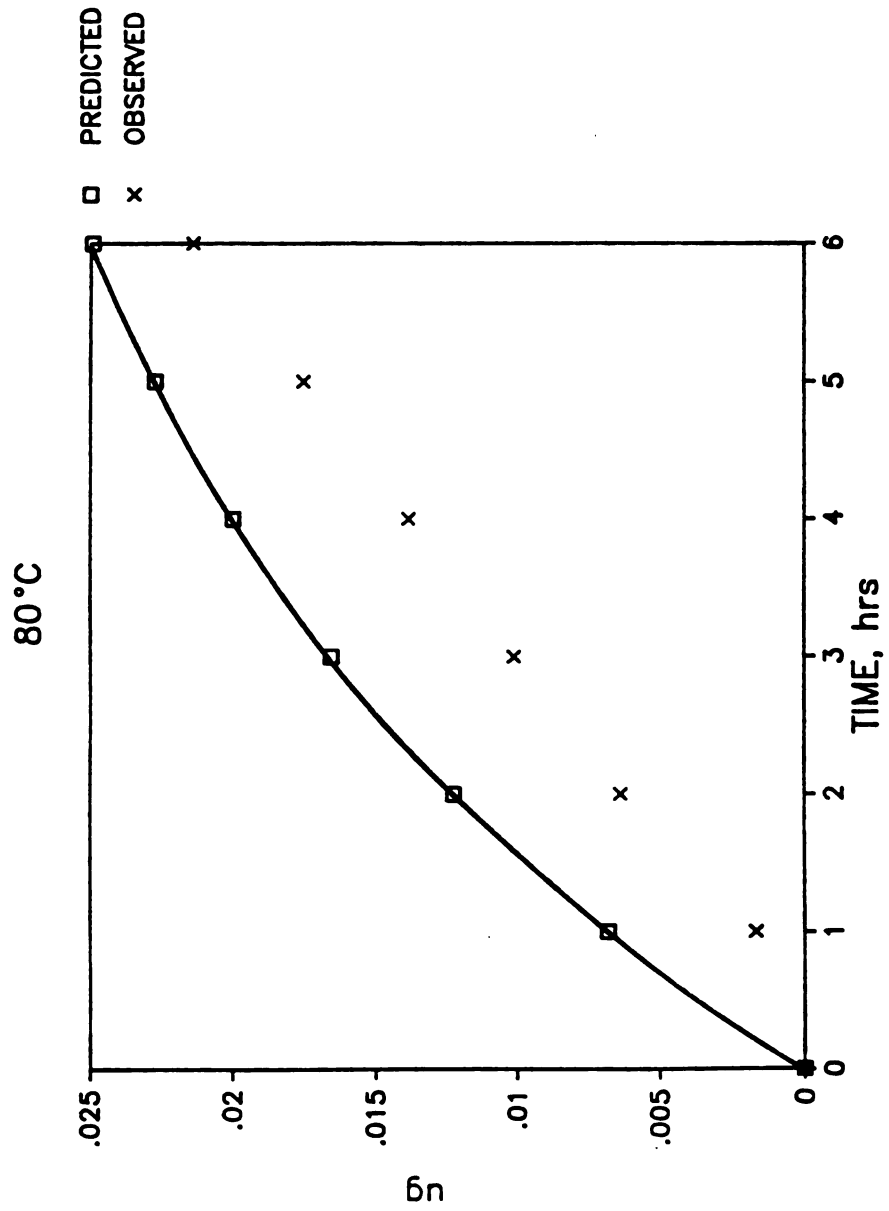
The production of dihydroactinidiolide is a function of the initial concentration of beta-carotene, absolute temperature ($^{\circ}\text{K}$) and time. Figures 8(a) to (d), show the formation of dihydroactinidiolide with time for each temperature investigated. Even though this technique for calculating rate of formation of volatiles at bond sites ignores contribution of non volatile degradation products and does not count for products formed from the oxidation of beta-carotene oxidation products, experimental results correlate well with predicted values and the model can be of use in the prediction of beta-carotene degradation and volatile degradation products formation.

Figure 8. Formation of dihydroactinidiolide during autoxidation of beta-carotene under excess oxygen at 25, 40, 60, and 80°C. Experimental and predicted values.









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CHAPTER III

COMPARISON OF METHODS FOR MEASURING ALPHA- AND BETA-CAROTENE CONCENTRATION IN CARROT PRODUCTS.

ABSTRACT

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COMPARISON OF METHODS FOR MEASURING ALPHA- AND BETA-CAROTENE CONCENTRATION IN CARROT PRODUCTS.

ABSTRACT

A comparison among an open column (Wallace, 1983), a reverse phase HPLC (Hsieh and Karel, 1983) and the official AOAC (1980) methods for the quantification of alpha- and beta-carotene was performed for dehydrated carrots. Samples were spiked with two levels of alpha- and beta-carotene and recoveries were determined for extraction and column chromatography. Both open column chromatography procedures resulted in substantial loss of both alpha- and beta-carotene. The AOAC method does not distinguish between alpha- and beta-carotene, therefore it cannot be used as a technique for vitamin activity. The HPLC method was the quickest, simplest and most reproducible method for analysing alpha- and beta-carotene and did not require saponification.

INTRODUCTION

The vitamin activity of fruits and vegetables is due to the presence of carotenoids. Of the numerous carotenoids known, only eighteen have pro-vitamin A activity, and each of them possesses a varying degree of activity (Sweeney and Marsh, 1971 and Underwood, 1984). In most vegetables and many fruits the majority of the pro-vitamin A activity is due to the trans form of alpha- and beta-carotene (Sweeney and Marsh, 1971). Alpha-carotene has only 50 - 54% of pro-vitamin A activity relative to that of beta-carotene (Thompson, 1986). Therefore, there is a need to have a simple and rapid method that would separate alpha- and beta-carotene yielding a reasonable value for vitamin A activity in fresh and processed fruits and vegetables. Such a method would be helpful in nutritional labeling and for nutritionists, plant biochemists and horticulturists.

The quantitative determination of carotenoids is very difficult because they are subject to isomerization and decomposition, requiring special care to minimize their exposure to air, light or reacting chemicals (De Ritter and Purcell, 1981). The methodologies available for determining carotenoids can be grouped into three chromatographic categories - open column, thin layer chromatography (TLC)

and high performance liquid chromatography (HPLC).

The open column techniques (Purcell and Walter, 1968; Rodriguez et al., 1976; Gebhardt et al., 1977 and AOAC, 1980) of which the AOAC is the most widely used, are very time consuming, not as quantitative as other methods due to numerous steps, and for most part cannot distinguish between alpha- and beta-carotene. The thin layer procedures are lengthy and the separation and quantitation of the carotenes is difficult (Davies, 1965 and Baloch et al., 1977). They also lack reproducibility, sensitivity and accurate quantitation (Zakaria and Simpson, 1979). TLC is not suitable for quantitative analysis because carotenes are destroyed by exposure to air (Thompson, 1986).

High performance liquid chromatography techniques are generally considered to be the quickest, simplest and most reproducible method for the separation and quantitation of carotenoids in fruits and vegetables (Sweeney and Marsh, 1971; Fiksdahl et al., 1978; Zakaria et al., 1979; Stancher and Zonta, 1982; Bushway and Wilson, 1982 and Hsieh and Karel, 1983). The most extensive system has been developed by Sweeney and Marsh (1971) for the determination carotene stereo-isomers. However, it is lengthy as it involves an initial open column step followed by two HPLC systems not available commercially. Many methods are reported, however many modifications and adaptations (solvent systems, for example) are required as these methods are difficult to reproduce (Stewart, 1977).

The main objective of this study was to evaluate current methodology for the separation and quantification of alpha- and beta-carotene. An open column method capable of separating alpha- and beta-carotene (Wallace, 1983) and a modified HPLC method (Hsieh and Karel, 1983) were compared to the official AOAC method.

EXPERIMENTAL PROCEDURES

Materials.

All-trans- alpha- and beta-carotene were obtained from Sigma Chemical Co., St. Louis, Mo. Instant carrots were purchased from local stores.

Standard solutions.

Stock standard solutions of all-trans- alpha- and beta-carotene were prepared by dissolving 20 mg of the reference standard in 5 ml of chloroform and diluting to 1000 ml of hexane. Working standards were prepared by diluting 0.3, 0.6, 1.2, 1.8, 2.4, 3.0, 3.6, and 4.2 ml of the alpha-carotene stock solution with 100 ml to give solutions containing 0.06, 0.12, 0.24, 0.36, 0.48, 0.60, 0.72, and 0.84 ug/ml. By diluting 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 7.0 ml of the beta-carotene stock solution to 100 ml, each solution contained 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4

ug/ml. Working standards were observed to be stable for one month at 4°C in the dark.

Methods of analysis.

Three methods were chosen: Method I: the AOAC method (AOAC, 1980) was chosen as it is the official method. Method II: an open column method capable of separating alpha- and beta-carotene as described by Wallace (personal communication, 1983). Method III: a modification of the method by Hsieh and Karel (1983). Table 1 describes key steps in each method. All analyses were done under subdued light.

METHOD I (AOAC)

The AOAC (1980) method for the determination of carotene in dried plant materials was followed. The sample was prepared by cold and hot saponification (AOAC, 1980). The standard curve was prepared by measurement of absorbance at 436 nm for the beta-carotene working solutions. The regression line obtained for this standard curve was $Y = 0.1684 x + 0.0016$ ($r^2 = 0.9999$).

METHOD II

Method II was an open column method capable of separating alpha- and beta-carotene, developed by Wallace (personal communication, 1983). The sample preparation consisted of: 2-5 g of sample, 100 ml of methanol, 10 ml of 60% KOH, 100 mg of BHT, and several glass beads were placed into 500 ml glass stoppered Erlenmeyer, topped with a reflux

Table 1. Diagram for the determination of carotenes by methods: I- AOAC (1980), II- open column chromatography (Wallace, 1983) and III- HPLC (Hsieh and Karel, 1983).

METHODS			
	I	II	III
EXTRACTION	hexane:acetone: ethanol:toluene (10:7:6:7)	methanol	acetone: pet. ether (1:1)
SAPONIFICATION	cold 20°C/16hr hot 56°C/20min 40% MeOH KOH	hot 60% KOH methanol 100°C/1hr	
SEPARATION	Open column activated magnesia: diatomaceous earth (1:1) 4% acetone in hexane	Open column MgO:5% hydrated Al ₂ O ₃ 4% acetone in hexane	HPLC C ₁₈ Bondapack acetonitrile chloroform (90:10) + 1ml MeOH
QUANTITATION	Absorbance 436 nm	Transmittance 450 nm	Absorbance 436 nm
IDENTITY	Total carotenoids	Alpha- & beta- carotene	Alpha- & beta- carotene

condenser and refluxed on steam bath for one hour. After cooling to room temperature, 50 ml of hexane were added, shaken vigorously, filtered in Buchner funnel (Whatman paper number 1), rinsed thoroughly and transferred to a 1000 ml separatory funnel. Distilled water (75 ml) was added to the separatory funnel. The hexane layer was drawn off and saved. The aqueous layer was extracted with five 50 ml portions of hexane. The hexane portions were pooled into a separatory funnel and washed free of alkali with five 100 ml portions of water. The extract was filtered through anhydrous sodium sulfate and concentrated to 100 ml by vacuum evaporation at 30°C in a Buchi rotary evaporator.

Purification and carotene separation: A 0.5 cm i.d. chromatographic column was packed under vacuum with a 1:1 mixture of magnesium oxide and 5% hydrated aluminum oxide to a height of 7.5 cm. A 0.5 cm layer of anhydrous sodium sulfate was added to the top of the column. The column was conditioned with hexane. Five ml of the sample extract was added and as it entered the column, 40 ml of 4% acetone in hexane were added. The separation of the carotenes was observed down the column. Alpha-carotene was a faint yellow band that travelled down most rapidly and was collected first. The second band, yellowish-red colored was beta-carotene. The bands were collected into a 10 and 25 ml flask respectively and completed to volume with 4% acetone in hexane. Transmittance at 450 nm was measured and alpha- and beta-carotene concentrations were calculated based on the

standard curves described by:

$$\text{Alpha-carotene: } Y = 2.181 - 0.022x \quad (r^2 = 0.999)$$

$$\text{Beta-carotene: } Y = 99.35 - 40.64x \quad (r^2 = 0.987)$$

METHOD III

Method III was an HPLC method described by Hsieh and Karel (1983) with some modifications: during extraction, 1 ml of water was added in order to improve recovery. Centrifugation of the sample at 650 rpm for 2 min after vortexing was useful in separating solids from the extract. During HPLC, a different solvent system gave better resolution on the separation of alpha- and beta-carotene. It consisted of acetonitrile:chloroform, 90:10 with 1 ml of methanol added to 250 ml of the mobile phase mixture. Alpha- and beta-carotene concentrations were calculated by linear regression alpha- and beta-carotene concentration and peak area:

$$\text{Alpha-carotene: } Y = 199.88x - 0.2907 \quad (r^2 = 0.9967)$$

$$\text{Beta-carotene: } Y = 173.28x - 0.4314 \quad (r^2 = 0.9984)$$

Recovery studies.

Recovery studies were performed by adding 0.20 and 0.50 mg of alpha- and beta-carotene to one gram of dehydrated carrots. Five replications were made at each spiking level and also before spiking.

Identification of HPLC peaks.

Identification of alpha- and beta-carotene were made by comparison of retention time with that of actual standards. Visible spectra from 300-600 nm were also taken on a Perkin-Elmer Lambda 4B UV/Vis C099-1301 spectrophotometer.

Analyses of experimental data.

All data collected were subjected to the ANOVA procedure of the MSTAT Statistical Package (1985). Comparison of means were done according to Duncan's method. Interpretation of all data was in accordance with Gill (1978).

RESULTS AND DISCUSSION

In the recovery study carried out using dehydrated carrots spiked with alpha- and beta-carotene, complete extraction of the pigment was easily determined by color removal. Table 2 shows the mean percent recoveries obtained at both spiking levels. Method I - cold extraction and method III gave best recoveries for both alpha- and beta-carotene. There was no significant difference ($p > 0.05$) between these two treatments, which may indicate that saponification was not required or that neutral fats, fatty acids and esters did not interfere in the determination of alpha- and beta-carotene in the dehydrated carrots. According to De Ritter and Purcell (1981), carotenoids in carrots, tomatoes and sweet potatoes are deposited as

Table 2. Recoveries^{1,2} of alpha- and beta-carotene during extraction from dehydrated carrots spiked with two levels of alpha- and beta-carotene, by the methods: I- AOAC (1980), II- open column chromatography (Wallace, 1983) and III- HPLC (Hsieh and Karel, 1983).

Extraction method	Percent Recovery	
	Alpha-carotene	Beta-carotene
Method I		
Cold	92.04 (13.48)	102.29 (5.09)
Hot	81.61 (10.87)	82.29 (13.67)
Method II	76.12 (9.73)	81.02 (13.14)
Method III	96.95 (5.02)	101.16 (4.42)

¹ Values represent means of five replications which were analysed in duplicate.

² Standard deviations are shown in parentheses.

crystals in a protein matrix. The use of acetone as extracting solvent in method III could denature the protein, releasing the carotenoids. The use of heat during saponification in methods I - hot extraction and method II resulted in a significant loss of both alpha- and beta-carotene. There was no significant difference ($p > 0.05$) among recoveries for alpha- and beta-carotene extraction by the three different methods, therefore, alpha- and beta-carotene behave in a similar way during any of the extraction methods.

In order to measure recovery of alpha- and beta-carotene during open column chromatography (methods I and II) samples spiked with standard and control were injected into the HPLC before and after open column chromatography. Mean percent recoveries are listed in Table 3. In both methods there is a significant loss of both alpha- and beta-carotene. Lack of reproducibility is also observed as indicated by the high standard deviation. The total recovery of alpha- and beta-carotene for each method is indicated in Table 4. There was no significant difference in the recovery of alpha- and beta-carotene in method I. In method II, recovery of alpha-carotene was significantly lower than recovery of beta-carotene. Its variability was also higher, as shown by the large value for the standard deviation. According to Baloch et al. (1977), adsorbents are usually destructive to carotenoids.

The HPLC method (method III) gave significantly better

Table 3. Recoveries^{1,2} of alpha- and beta-carotene during column chromatography, of extracts from dehydrated carrots spiked with two levels of alpha- and beta-carotene, by methods: I- AOAC (1980) and II- Open column chromatography (Wallace, 1983).

Method	Percent Recovery	
	Alpha-carotene	Beta-carotene
Method I	87.38 (5.00)	85.46 (6.91)
Method II	79.02 (10.13)	97.16 (6.40)

¹ Values represent means of five replications which were analysed in duplicate.

² Standard deviations are shown in parentheses.

Table 4. Total recoveries^{1,2} of alpha- and beta-carotene during extraction and chromatography of dehydrated carrots spiked with two levels of alpha- and beta-carotene, by the methods: I- AOAC (1980), II- open column chromatography (Wallace, 1983) and III- HPLC (Hsieh and Karel, 1983).

Method	Percent Recovery	
	Alpha-carotene	Beta-carotene
Method I		
Hot	71.19 (11.90)	70.25 (12.70)
Cold	80.65 (12.39)	87.55 (9.92)
Method II	62.24 (9.06)	81.16 (7.49)
Method III	96.95 (5.02)	101.16 (4.42)

¹ Values represent means of five replications which were analysed in duplicate.

² Standard deviations are shown in parentheses.

recoveries and lowest variability. In Figure 1, there is a chromatogram showing the separation of alpha- and beta-carotene in dehydrated carrots. The peaks corresponding to alpha- and beta-carotene are designated by numbers 1 and 2, respectively. Although 100% resolution is not achieved (actual resolution is approximately 91.6%), alpha- and beta-carotene are still easily and accurately quantified. The HPLC method was very sensitive, being able to detect concentrations as low as 0.1 ug/ml of beta-carotene and 0.06 ug/ml of alpha-carotene. The repeatability from injection to injection was good. The separation takes approximately 15 minutes. The entire procedure, including sample preparation takes 30 - 40 minutes and thus is a faster procedure than the other methods evaluated.

Table 5 shows the values obtained for alpha- and beta-carotene in dehydrated carrots by the three methods. The values are adjusted for recovery according to Table 4. There was significant difference ($p < 0.05$) among total carotene content obtained by each method. Table 6 shows values obtained when samples with higher moisture content (carrot puree and fresh carrots) are analysed by these methods. There was no significant difference ($p < 0.05$) in the total carotene among methods for the carrot puree. The higher water content in these samples seemed to interfere with extraction by the HPLC procedure affecting its recovery. An increased ratio of acetone in the extraction solution, could improve recovery.

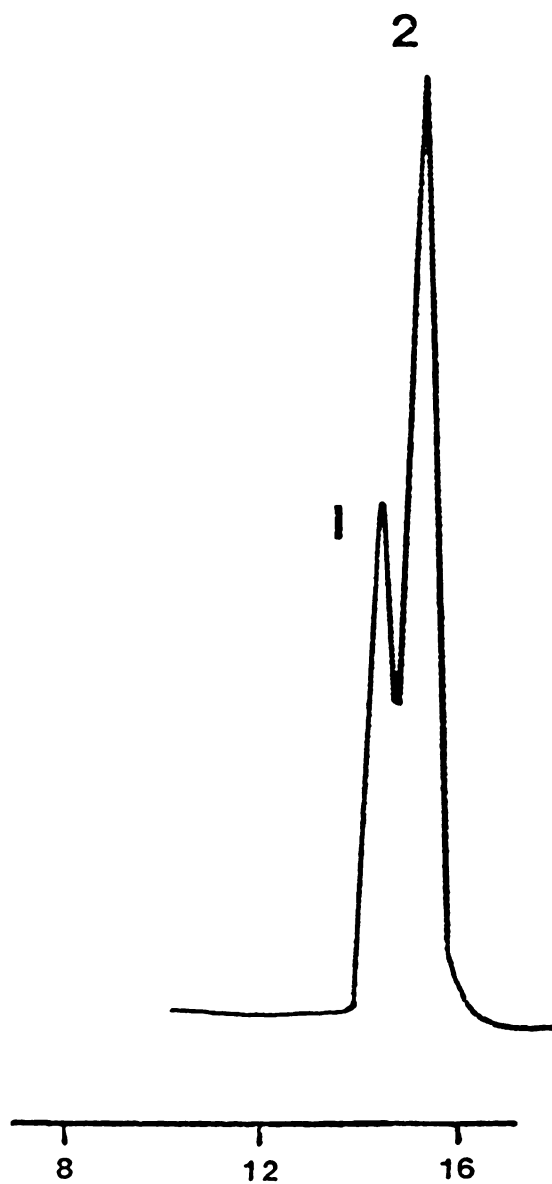


Figure 1. HPLC separation of (1) alpha-carotene and (2) beta-carotene by C₁₈ Bondapack with solvent system of acetonitrile:chloroform (90:10) and 1ml of methanol at 1ml/min, and detector set at 436 nm.

Table 5. Alpha- and beta-carotene content*,^{1,2} (mg/100g) in dehydrated carrots by methods: I- AOAC (1980), II- open column chromatography (Wallace, 1983) and III- HPLC (Hsieh and Karel, 1983).

Sample	Method		
	I	II	III
Instant carrot # 1			
Alpha		13.86 (1.18)	19.72 (1.86)
Beta		28.28 (3.55)	40.11 (1.61)
Total	39.39 (4.19)	42.14 (4.76)	58.82 (3.46)
Instant carrot # 2			
Alpha		27.42 (1.60)	32.13 (3.28)
Beta		55.18 (2.57)	73.56 (4.83)
Total	113.14 (2.62)	82.40 (2.60)	105.69 (7.51)

* Values are recovery adjusted according to Table 3.

¹ Values represent means of three replications which were analysed in duplicate.

² Standard deviations are shown in parentheses.

Table 6. Alpha- and beta-carotene content*,^{1,2} (mg/100g) in carrot puree and fresh carrots by methods: I- AOAC (1980), II- open column chromatography (Wallace, 1983) and III- HPLC (Hsieh and Karel, 1983).

Sample	Method		
	I	II	III
Puree # 1			
Alpha		4.05 (0.41)	3.06 (0.28)
Beta		6.22 (0.60)	6.03 (0.73)
Total	9.18 (0.88)	10.27 (1.01)	9.09 (1.01)
Puree # 2			
Alpha		2.17 (0.05)	1.94 (1.31)
Beta		4.62 (0.41)	4.74 (1.07)
Total	6.51 (0.68)	6.81 (0.43)	6.69 (1.31)
Fresh carrots			
Alpha		2.46 (0.04)	2.64 (0.10)
Beta		4.32 (0.19)	5.72 (0.21)
Total	5.50 (0.57)	6.78 (0.21)	8.36 (0.23)

* Values are recovery adjusted according to Table 3.

¹ Values represent means of three replications which were analysed in duplicate.

² Standard deviations are shown in parentheses.

CONCLUSION

The AOAC method, method I, does not distinguish between alpha- and beta-carotene. According to Bushway and Wilson (1982), other carotenoids such as lycopene and monohydroxy carotenoids, are also eluted from the column and are quantified along with carotenes. In the case of carrots, alpha- and beta- carotene constitute the major fraction of carotenoids (Ogunlesi and Lee, 1978).

Method II gave results that were significantly different from method III for both alpha- and beta-carotene. It is a lengthy procedure. However it should be considered where HPLC is not available as long as the recovery factor is determined and used. The use of a less severe saponification procedure could improve its recovery. The HPLC method, method III, was the quickest, simplest and most reproducible method. It would constitute a useful tool in nutritional labelling and in studying effects of different cultural and processing treatments on these two carotenoids.

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CHAPTER IV

KINETICS OF BETA-CAROTENE OXIDATION. PART III.

BETA-CAROTENE DEGRADATION AND VOLATILE

FORMATION IN DEHYDRATED CARROTS.

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KINETICS OF BETA-CAROTENE OXIDATION. PART III.

BETA-CAROTENE DEGRADATION AND VOLATILE FORMATION IN DEHYDRATED CARROTS.

ABSTRACT

The degradation of alpha- and beta-carotene and production of volatile degradation products were investigated in dehydrated carrots at temperatures of 20, 40 and 60°C under excess oxygen in order to investigate the applicability of the kinetic model approach (developed in Chapter II) to a food system. The first order model describes the rate of alpha- and beta-carotene losses over the temperature range investigated. The rate of beta-carotene loss in dehydrated carrots was much lower than that observed in model systems. The rate of beta-carotene loss was higher than the rate of alpha-carotene loss.

Among volatile degradation products previously identified in the model system (Chapter II) 2,2,6-trimethyl cyclohexane carboxaldehyde, alpha-isophorone, beta-cyclocitral, alpha-ionone, beta-ionone, 5,6-epoxy beta-ionone and dihydroactinidiolide were present and represented

40 to 50 % of the total volatile fraction from autoxidation of beta-carotene in dehydrated carrots. The mathematical model correlating beta-carotene degradation and production of dihydroactinidiolide as a function of temperature and storage time was a satisfactory method for monitoring beta-carotene loss in this food system.

INTRODUCTION

Carotenoids are extremely sensitive to oxygen, light and heat, and degradation will lead to loss of color, loss of vitamin A activity and development of off-flavor (Archer and Tannenbaum, 1979). Dehydrated foods are most susceptible to loss of carotenoids because of their tendency to undergo oxidation (Labuza et al., 1970).

Degradation of carotenoids has been reported to play an important role in determining the overall storage life of dehydrated carrots. Falconer et al. (1964) reported a significant correlation between the development of off-flavors and color loss in freeze dried carrot powder stored at 18°C. The off-flavors, described as violet- and hay-like became noticeable (and the dehydrated product unacceptable) when 20-45 % of the total carotenoid had been destroyed (Tomkins et al., 1944). Falconer et al. (1964) observed that even when 5% color loss had occurred, off-flavor was detected. This violet-like aroma has been suggested to be due to the formation of beta-ionone (Tomkins et al., 1944; Falconer et al., 1964; Ayers et al., 1964) and alpha-ionone and 5,6-epoxy beta-ionone (Ayers et al., 1964).

The degradation of carotenoids in dehydrated carrots has been investigated with respect to the effect of water

activity (Tomkins et al., 1944; Arya et al., 1979), oxygen pressure (Stephens and McLemore, 1969), storage temperature (Stephens and McLemore, 1969) and additives (Tomkins et al., 1944; Feinberg et al., 1964; Stephens and McLemore, 1969; Nutting et al., 1970; Ellis et al., 1970; Roberts and McWeeny, 1972; Mohammad et al., 1976; Speck et al., 1977; Arya et al., 1979). Nevertheless, results from these investigations are rather conflicting. In all of these investigations, carotenoid concentrations were determined by measuring absorbance at 450 nm, therefore total carotenoids were evaluated. Alpha- and beta-carotene constitute 20 and 70%, respectively, of the total carotenoid content in dehydrated carrots. No one has investigated the stability of individual carotenoids in dehydrated carrots.

In Chapter 2, the mechanism and the general kinetic principle of the degradation of beta-carotene was investigated in a model system simulating the food. Beta-carotene loss was observed to follow a first order degradation. It was also observed that beta-carotene degradation correlated well with dihydroactinidiolide formation. The main objective of this study was to investigate the applicability of the kinetics developed in Chapter 2 to a food system.

EXPERIMENTAL PROCEDURES

Material.

Instant carrots were obtained from local stores. Alpha- and beta-carotene standards were purchased from Sigma Chemicals, St. Louis, MO. Volatile standards were purchased from Sigma Chemicals, (St. Louis, MO), Aldrich Chemicals (Milwaukee, WI) and ICN Chemicals (Plainview, NY). Beta-isophorone, beta-cyclocitral, 5,6-epoxy beta-ionone and dihydroactinidiolide were synthesized according to the Chapter I.

Effect of temperature.

Forty grams of the dehydrated carrots were placed in a round bottom flask, covered with aluminum foil, and immersed in a paraffin bath at temperatures of 20, 40 and 60°C. An oxygen tank was attached to the system, furnishing oxygen (99.8% pure) at a flow of 10 ml/min. Periodically, samples were withdrawn from the reaction vessel and analysed for beta-carotene content and volatile degradation products.

Analysis of alpha- and beta-carotene.

Alpha- and beta-carotene were extracted and analyzed by C₁₈ Bondapak reverse phase HPLC as described in Chapter III.

Volatile degradation products.

The volatiles formed by the oxidation of beta-carotene were collected by a Tenax GC trap system, separated and quantitated by GC and identified by GC/MS as described in Chapter II.

RESULTS AND DISCUSSION

Stability of alpha- and beta-carotene.

The content of alpha- and beta-carotene in the dehydrated carrots used in this investigation was 22.22 and 72.66 mg/100g, respectively. The moisture content of the dehydrated carrots was 4.28%.

Figures 1 and 2 show the relationship between alpha- and beta-carotene loss and reaction time at 20, 40 and 60°C under oxygen. The loss of alpha- and beta-carotene at all temperatures investigated could be described by a first order reaction, as it gave very good correlation for linear regression. The sample stored at 20°C showed an induction period not observed at higher temperatures or in model system studies (Chapter II).

Calculated first order rate constants for each temperature are indicated in Table 1. The fit of the first order model to the food system data was about as good as the fit for the model system, based on the r^2 data. However, consistent bias is shown at higher temperatures. The bias could be explained in part by the presence of free radical

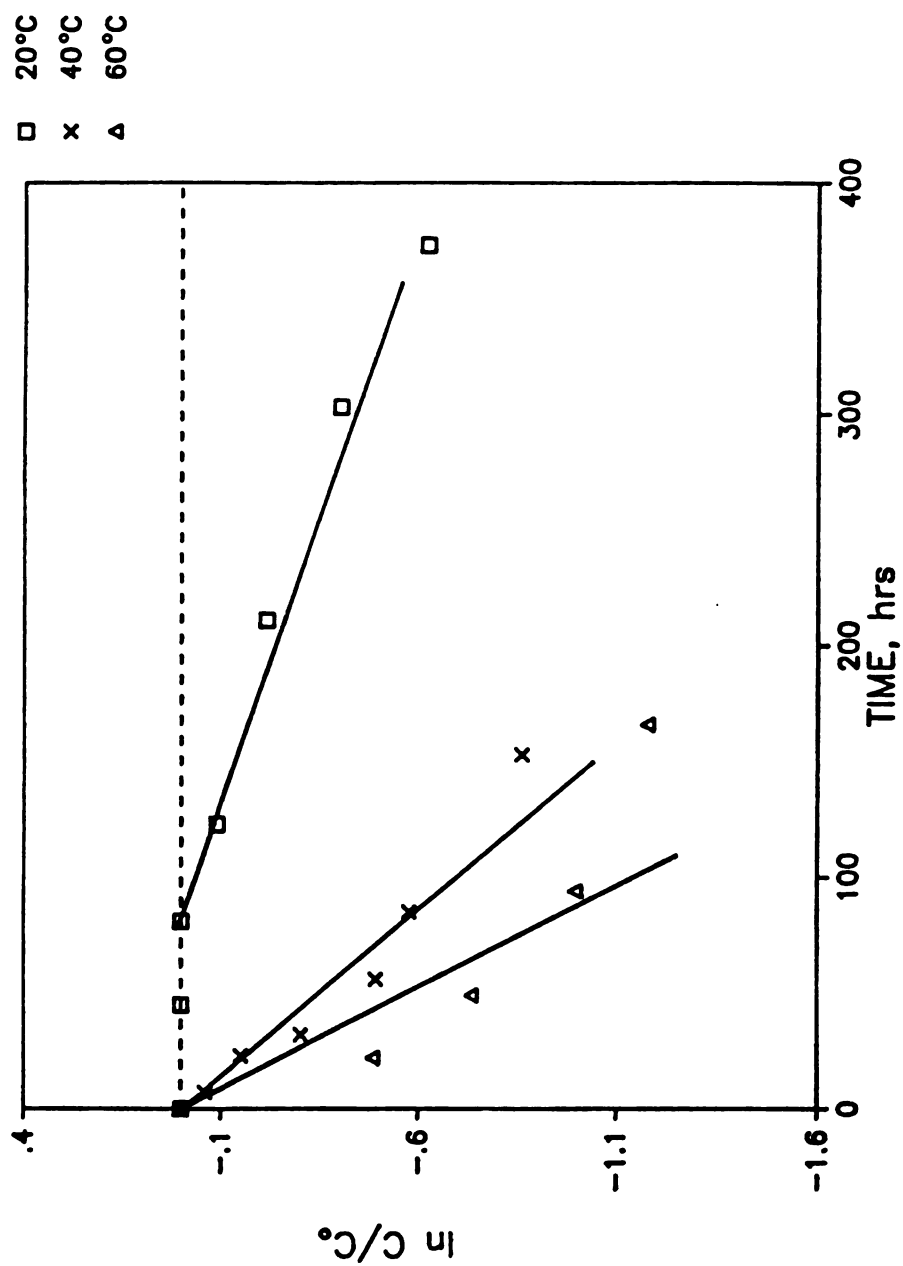


Figure 1. Autoxidation of alpha-carotene in dehydrated carrots under excess oxygen at 20, 40 and 60°C.

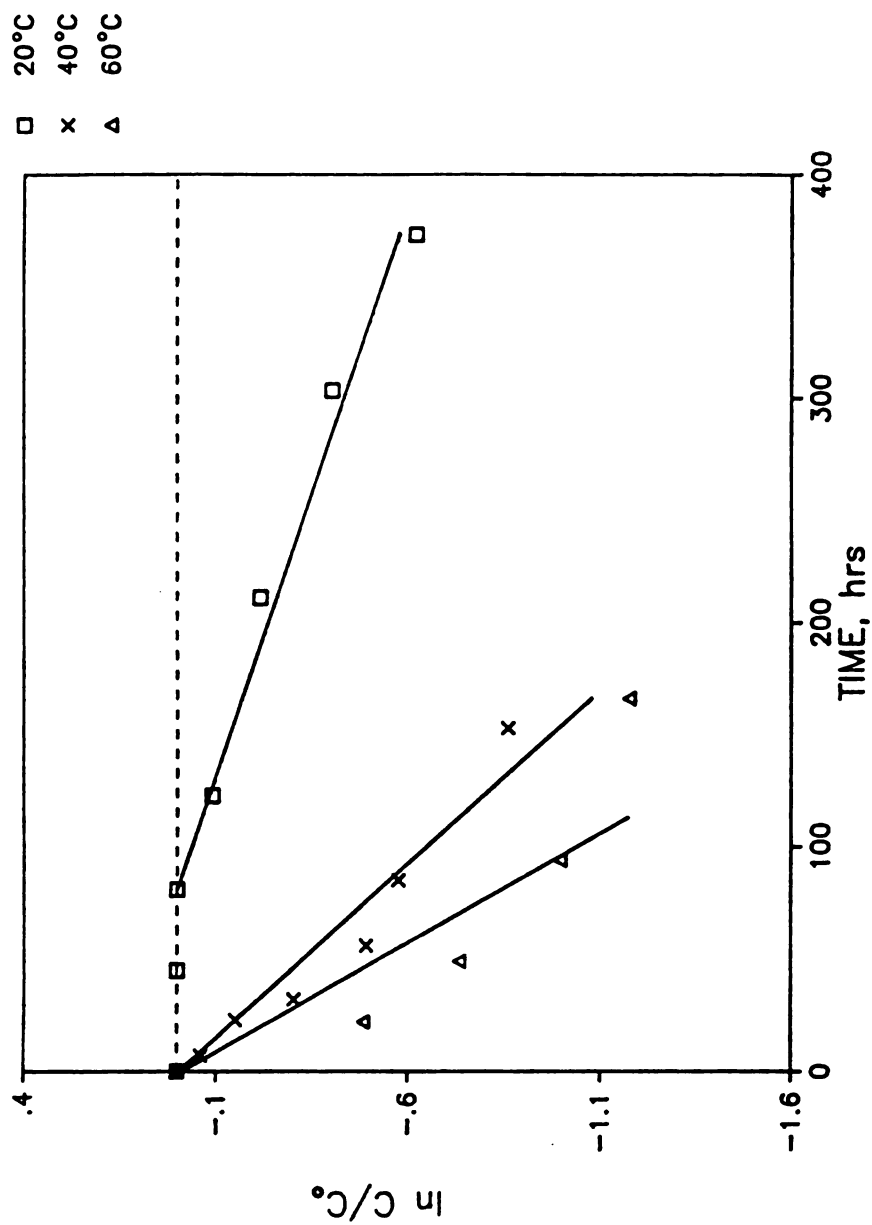


Figure 2. Autoxidation of beta-carotene in dehydrated carrots under excess oxygen at 20, 40 and 60°C.

Table 1. First order rate constants and activation energies for alpha- and beta-carotene loss during autoxidation of dehydrated carrots at 20, 40 and 60°C.

Autoxidation at T°C		Rate constants (hr ⁻¹)	
		Alpha-carotene	Beta-carotene
20	k	.00158	0.002027
		(0.0003)	(0.00018)
	r ²	.9586	.9678
40	k	0.0046	0.0057
		(0.0003)	(0.0004)
	r ²	.9657	.9417
60	k	0.02033	0.0148
		(0.0036)	(0.0017)
	r ²	.9932	.9319
Ea Kcal/ mol		12.22	9.56
Frequency factor		2.17 x 10 ⁶	3.03 x 10 ⁴

¹ Values represent means of three replications which were analyzed in duplicate.

² Standard deviations of the rate constants are shown in parentheses.

inhibitors or traps in the dehydrated carrots. The rate constants for the degradation of beta-carotene in model systems are significantly higher than experimental values for dehydrated carrots. Apparently, there are factors in the food systems reducing the rate of oxidation. The rate of beta-carotene loss is higher than that for alpha-carotene at 20 and 40°C, however at 60°C, the rate of alpha-carotene degradation becomes higher. In contradiction to these results, Ayers et al. (1964) observed that the stability of the alpha-ionone ring was lower than that of the beta-ionone ring as they observed equal ratios of alpha- and beta-ionone from a sample containing 20:70 alpha-:beta-carotene. However, during oxidation, beta-ionone itself is autoxidized into 5,6-epoxy beta-ionone, resulting in decreased beta-ionone levels (Chapter I).

The activation energy (Figure 3) obtained in the range of 20-60°C was of 12.22 ± 1.64 and 9.56 ± 0.13 kcal/mol for alpha- and beta-carotene respectively. The loss of beta-carotene is therefore the favored.

Volatile degradation products.

At all temperatures, seven volatile degradation products common to those identified from the oxidation of beta-carotene in a model system accounted for 40 to 50% of the total volatile fraction: 2,2,6-trimethyl cyclohexane carboxaldehyde, alpha-isophorone, beta-cyclocitral, alpha-ionone, beta-ionone, 5,6-epoxy beta-ionone and

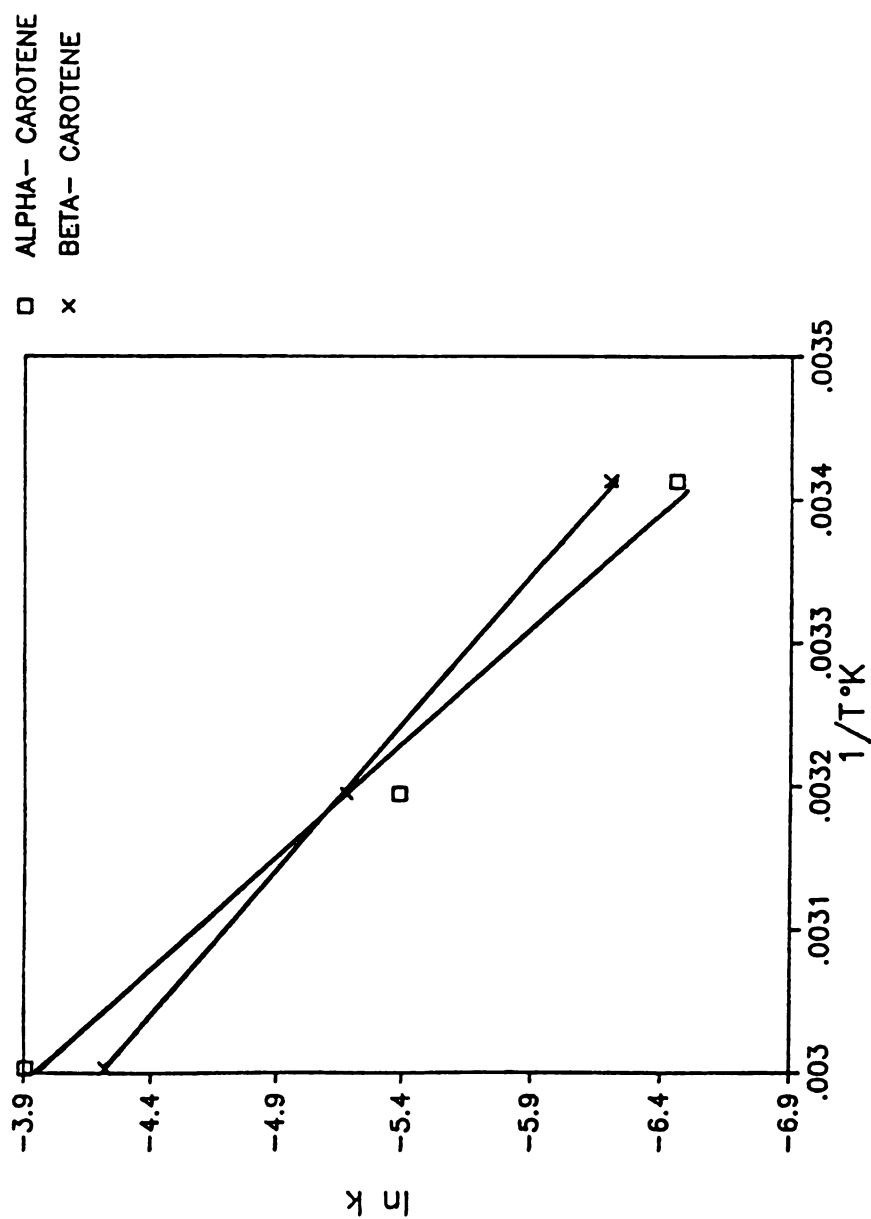


Figure 3. Activation energy for the autoxidation of alpha- and beta-carotene in dehydrated carrots at 20, 40 and 60°C under excess oxygen by the first order reaction.

dihydroactinidiolide. Several other compounds that were not common to those from beta-carotene autoxidation in the model system were present, however their identification was not pursued.

At all investigated temperatures, in an analogous way to the model system, there was a significant continuous increase in the contribution of 5,6-epoxy beta-ionone and dihydroactinidiolide to the total volatile fraction with oxidation time. However, a decrease was observed for 2,2,6-trimethyl cyclohexane carboxaldehyde, beta-cyclocitral and beta-ionone. The contribution of alpha-ionone to the flavor profile was more accentuated than in beta-carotene model systems and it could be due to the autoxidation of alpha-carotene. Since alpha-ionone, beta-ionone, 5,6-epoxy beta-ionone and dihydroactinidiolide have been described as having hay like flavor, the control of their presence is desirable. Table 2 shows the beta-carotene volatile degradation products observed during the autoxidation of dehydrated carrots.

Kinetics for beta-carotene degradation into volatile degradation products.

Since beta-carotene autoxidation in dehydrated carrots fits a first order kinetics and the plots of P_1 vs P_3 are linear (see Figure 4), the model proposed by Levenspiel (1972) can be applied. P_3 was chosen as a reference as it behaves in a similar way for all temperatures and it is

Table 2. Compounds formed from cleavage of beta-carotene at bonds 6-7, 7-8, 8-9 and 9-10 during autoxidation of dehydrated carrots under excess oxygen at 20, 40 and 60°C.

Bond	Compounds
6-7	alpha-isophorone
7-8	2,2,6-trimethyl cyclohexane 1 carboxaldehyde beta-cyclocitral
8-9	dihydroactinidiolide
9-10	alpha-ionone beta-ionone 5,6-epoxy beta-ionone

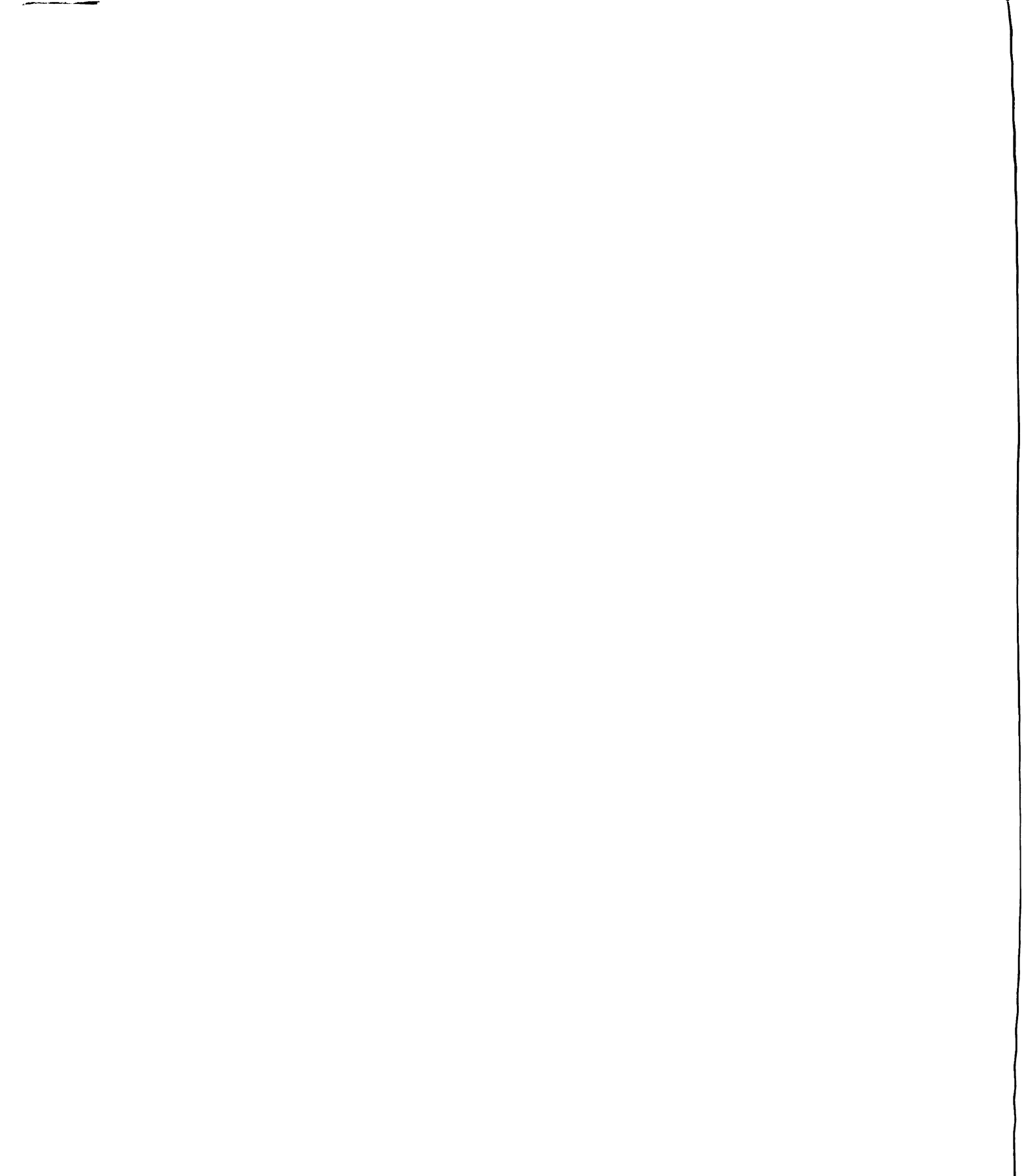
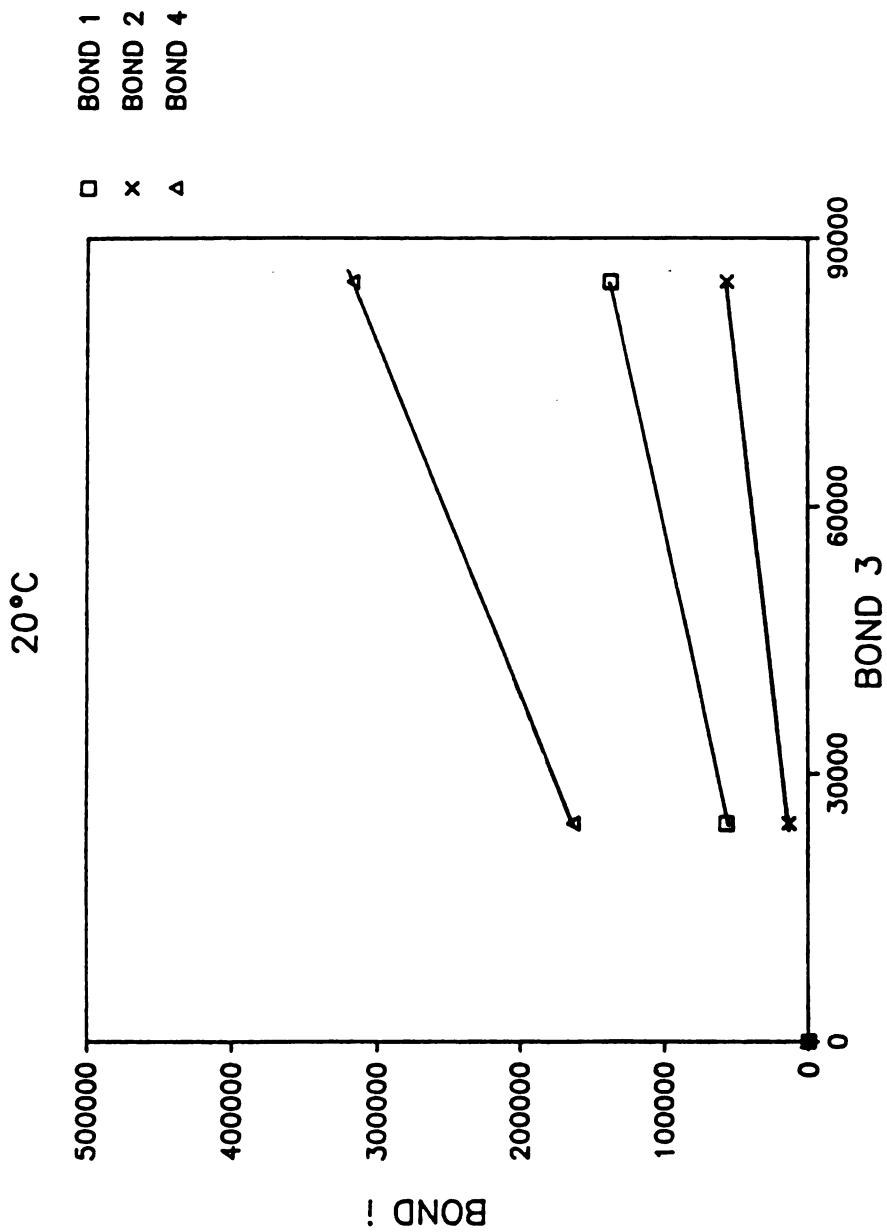
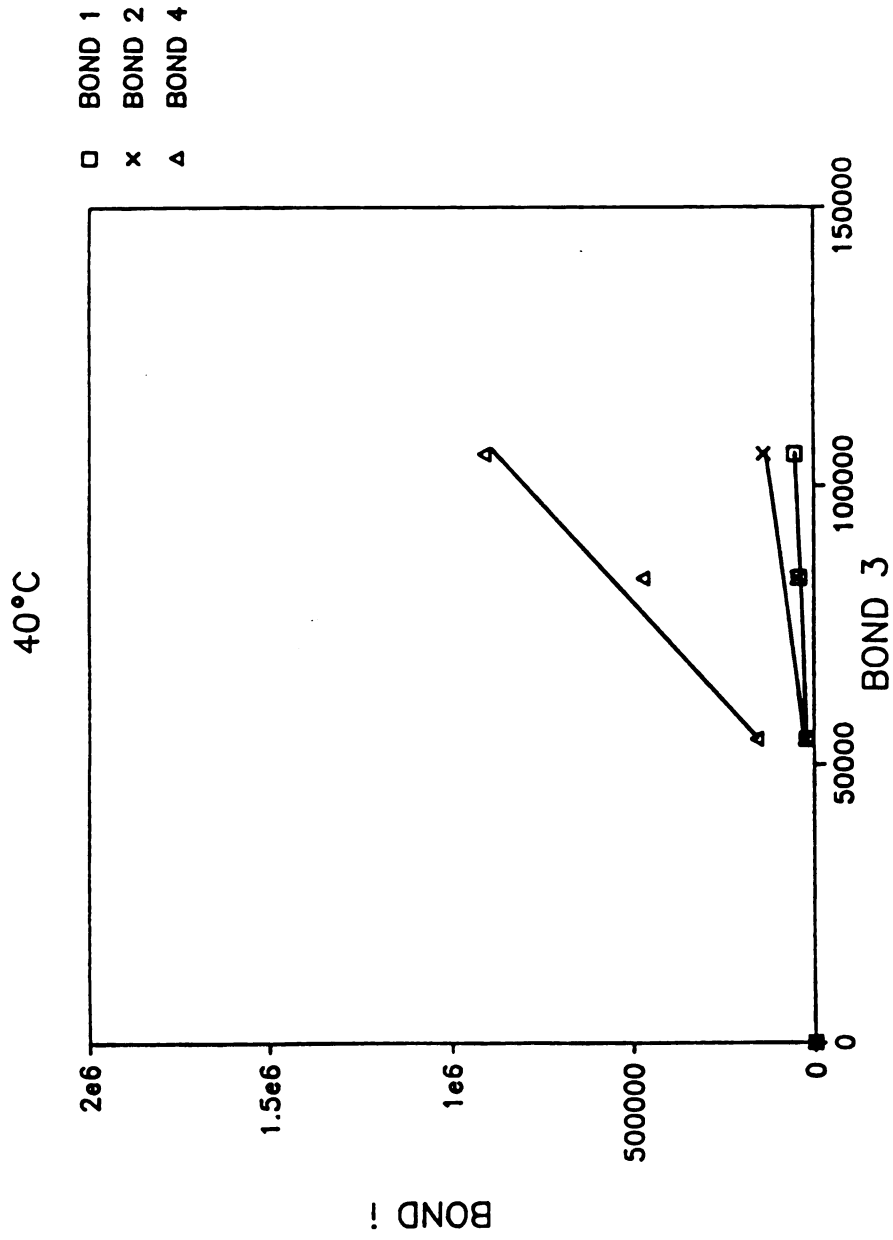
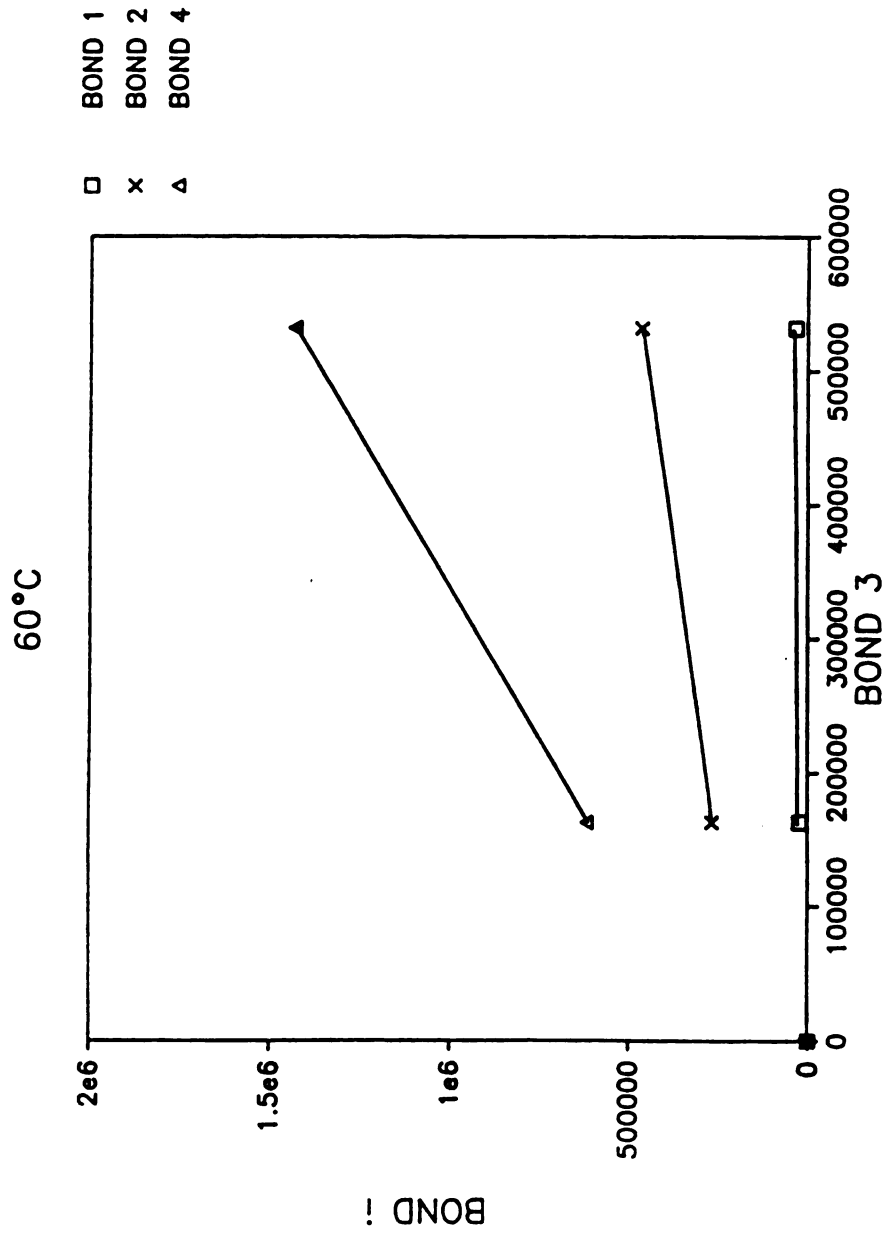


Figure 4. Plot of P_1 versus P_3 during autoxidation of beta-carotene in dehydrated carrots under excess oxygen at 20, 40 and 60°C.







represented by only one compound: dihydroactinidiolide.

Calculated rate constants for the formation of volatile degradation products according to Levenspiel's approach, are listed in Table 3. Figure 5 and Table 3 show the temperature dependency of the rate of formation of volatile degradation products. The increase in temperature reduces the cleavage at bond 6-7.

Using activation energy values and frequency factors (Tables 1 and 4) in the Equation 23 of Chapter II, we can predict volatile formation. According to Chapter II, the monitoring of dihydroactinidiolide (as P_3) would be a good reference for monitoring beta-carotene oxidation. The concentration of dihydroactinidiolide during oxidation of beta-carotene can be described by:

$$P_3 = 49.5C_0 \exp\left(-\frac{3670}{RT}\right) [1 - \exp(-(30300 \exp(-\frac{9560}{RT}))t)]$$

(1)

As one can see, the production of dihydroactinidiolide will be a function of the initial concentration of beta-carotene, absolute temperature (°K) and time. Figures 6 (a) to (c), show the formation of dihydroactinidiolide with time for each temperature investigated. Even though this technique for calculating " k_1 ", rate of formation of volatiles at bond sites, ignores contribution of non volatile degradation products and assumes beta-carotene to be the only source of dihydroactinidiolide, experimental results correlate well with predicted values. The only

Table 3. First order rate constants for the formation of breakdown products from bonds 6-7, 7-8, 8-9 and 9-10 of beta-carotene in dehydrated carrots under excess oxygen at 20, 40 and 60°C.

Bond	Rate Constant (hr^{-1}) at T°C		
	20	40	60
6 - 7	0.00041 (0.00004)	0.00017 (0.00005)	0.00012 (0.00006)
7 - 8	0.00029 (0.00002)	0.00095 (0.00024)	0.0022 (0.0008)
8 - 9	0.00028 (0.00014)	0.00084 (0.0004)	0.0041 (0.0008)
9 - 10	0.0010 (0.00009)	0.0037 (0.00017)	0.0084 (0.0015)

¹ Values represent means of three replications analyzed in duplicate.

² Standard deviations are shown in parentheses.

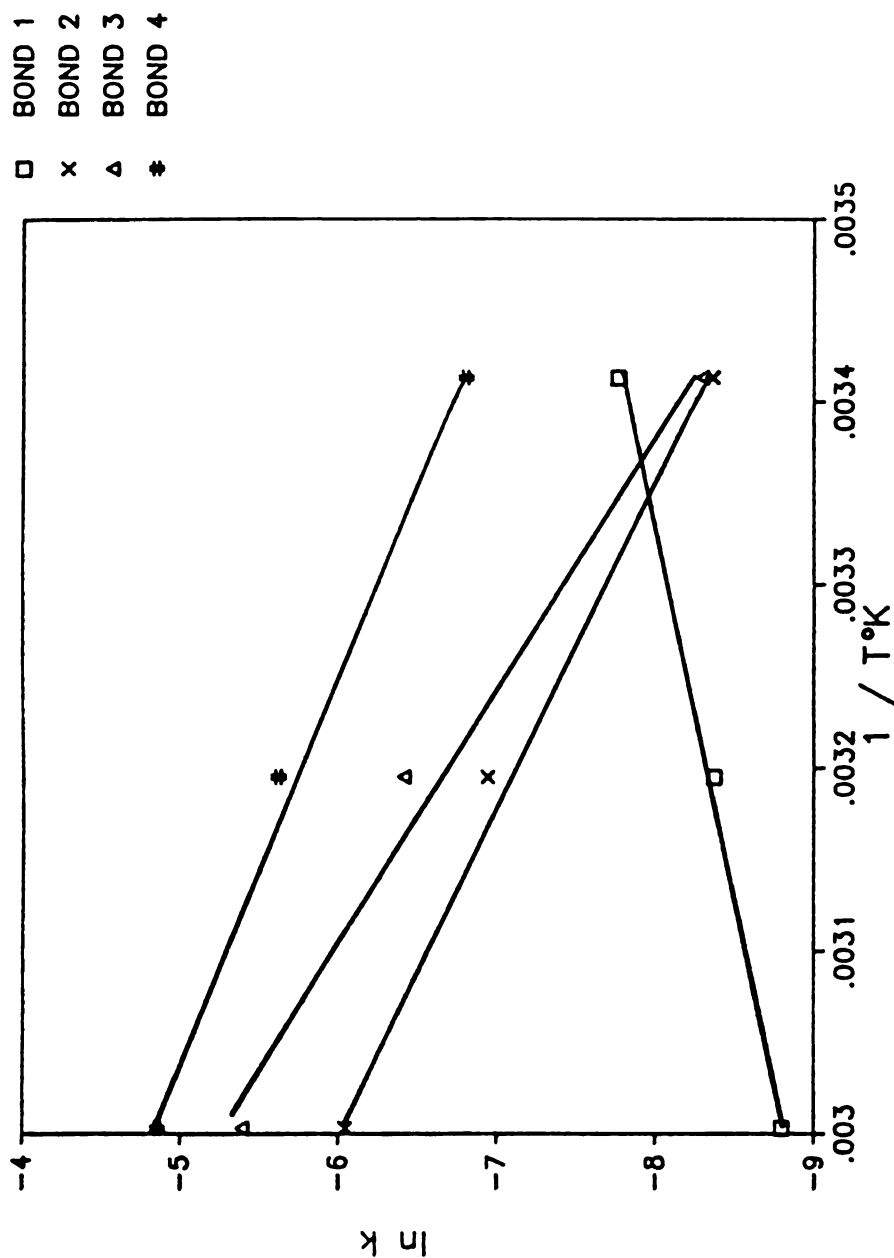


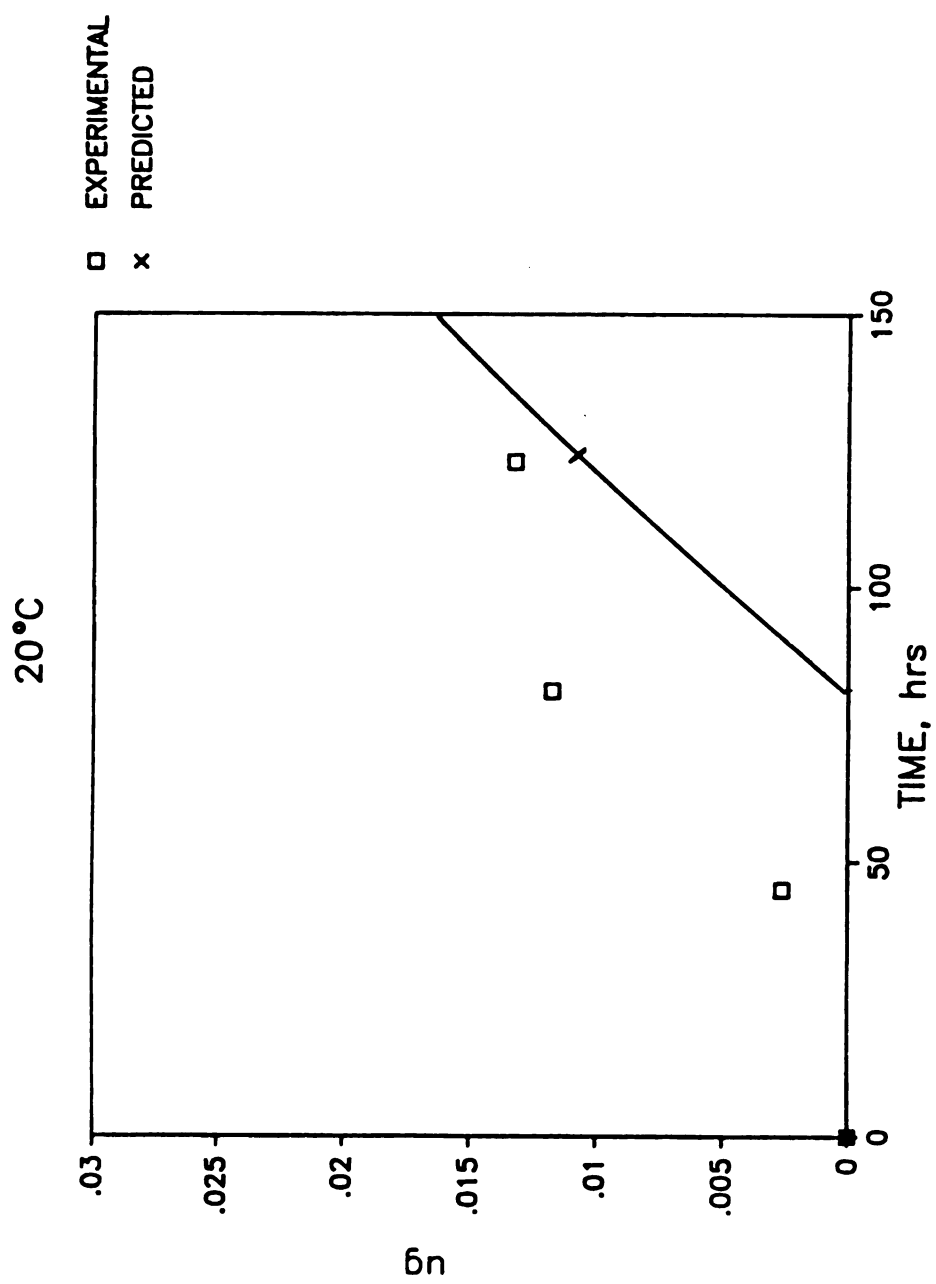
Figure 5. Arrhenius plot for the formation of products from cleavage at bonds 6-7, 7-8, 8-9 and 9-10 of beta-carotene in dehydrated carrots under excess oxygen at 20, 40 and 60°C.

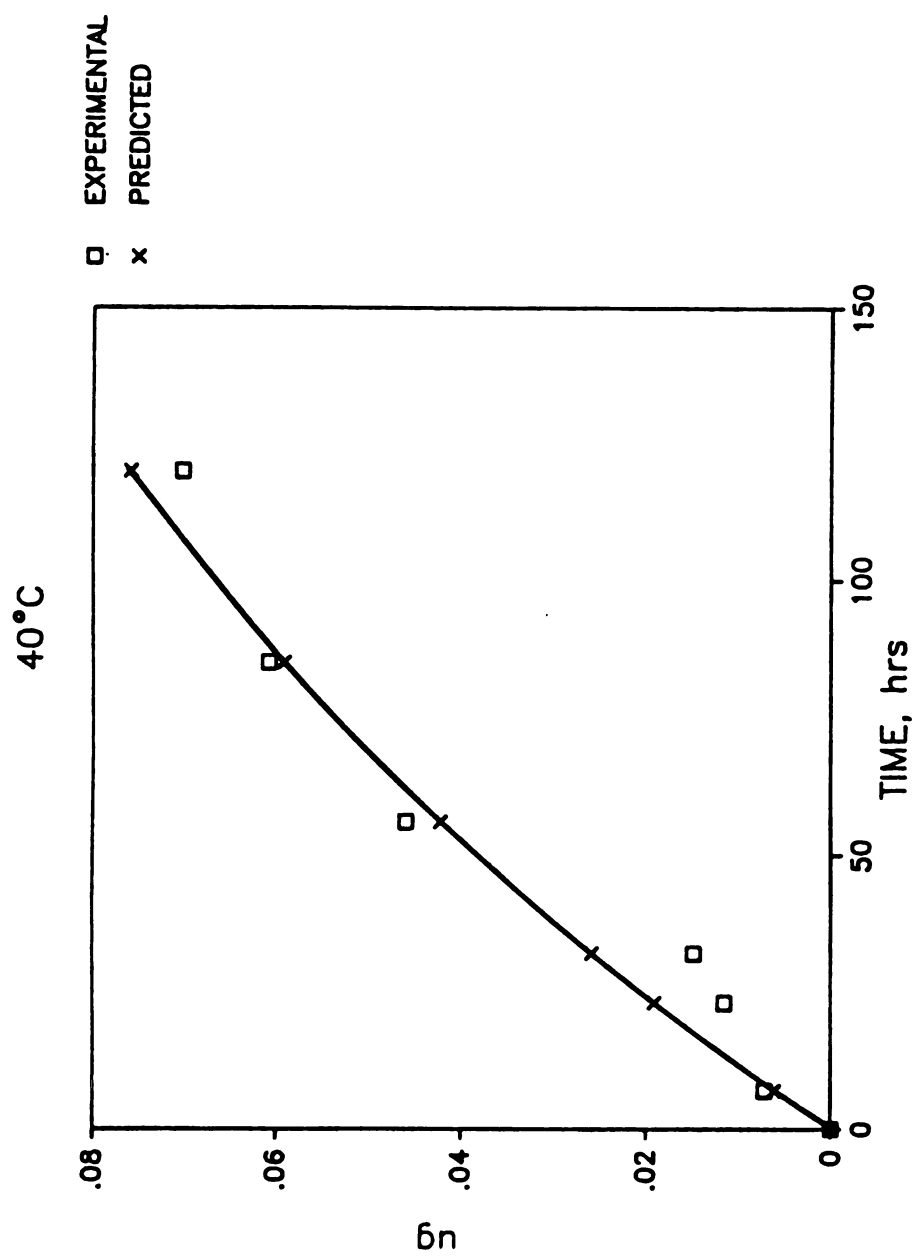
Table 4. Activation energy¹ for the formation of degradation products from bonds 6-7, 7-8, 8-9 and 9-10 of beta-carotene in dehydrated carrots under excess oxygen at 20, 40 and 60°C.

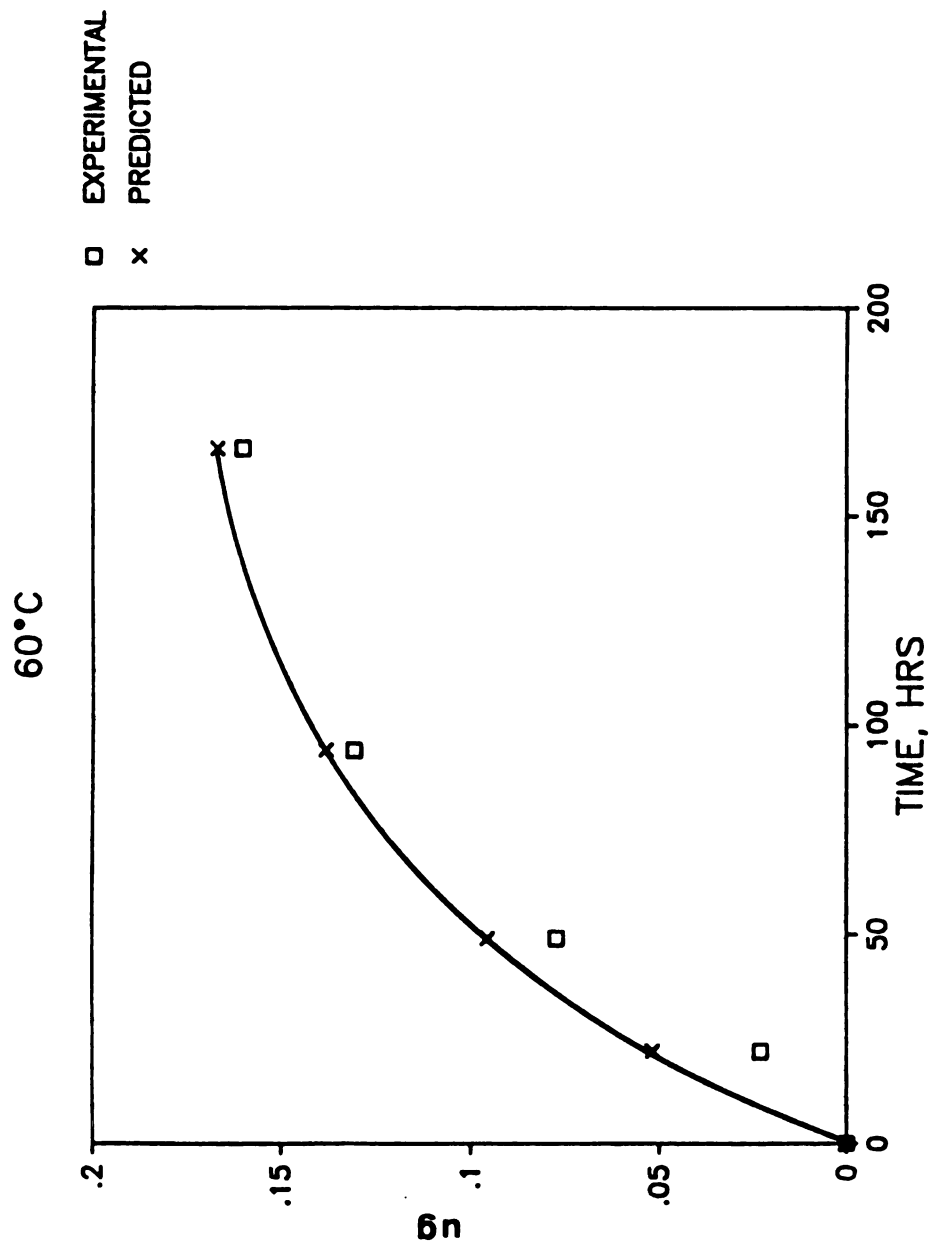
Bond	Activation Energy	Frequency factor
	Kcal/mol	
6-7	6.31 (.8311)	7.0×10^{-9}
7-8	9.54 (.9887)	4.3×10^3
8-9	13.23 (.9573)	1.5×10^6
9-10	10.05 (.9897)	3.8×10^4

¹ Correlations for linear regression are shown in parentheses.

Figure 6. Formation of dihydroactinidiolide during autoxidation of beta-carotene in dehydrated carrots under excess oxygen at 20, 40 and 60°C. Experimental and predicted values.







exception is at 20°C where predicted values were higher than observed values. That could be due to the fact that the model developed does not account for the induction period observed for loss of beta-carotene at 20°C.

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CONCLUSIONS

The conclusions reached as a result of this investigation are summarized as follows:

1. The determination of beta- carotene by simple measurement of absorbance at 450 nm is not a good procedure for monitoring beta-carotene loss as oxidation products also absorb at similar region of the visible spectra.

2. A normal phase HPLC system, using a gradient of acetone in hexane, was capable of separating beta-carotene from its degradation products. It can be used to monitor beta-carotene loss in model or food systems.

3. A reverse phase HPLC method capable of separating alpha- and beta-carotene was improved and compared to an open column method Wallace (1983) and to the official AOAC method. It was observed to be the simplest, fastest and most reproducible method for the separation of alpha- and beta-carotene from food systems.

4. In the analysis of the kinetics of beta-carotene oxidation in the presence of excess oxygen in the model system, the first order approach was the most satisfactory for the loss of beta-carotene over the temperature range investigated.

5. Volatile oxidation products comprised mainly of C_9 , C_{10} , C_{11} and C_{13} compounds originated from cleavage of bonds 6-7, 7-8, 8-9 and 9-10 from beta-carotene.

6. Alpha-isophorone, 2,2,6 trimethyl cyclohexane 1-carboxaldehyde, beta-cyclocitral, beta-ionone, 5,6 epoxy beta-ionone and dihydroactinidiolide represented 60 to 80% of the total volatile products in the model system.

7. First order models adequately describe beta-carotene loss and product formation for degradation with excess oxygen over the temperature range of 25 - 80°C.

8. The production of dihydroactinidiolide correlated well with beta-carotene loss in the Avicel model system during autoxidation. This correlation was satisfactory up to 50% loss of beta-carotene.

9. During oxidation of dehydrated carrots in excess oxygen at 20, 40 and 60°C, both alpha- and beta-carotene loss followed a first order reaction. The loss of beta-carotene was higher than the loss of alpha-carotene.

10. The production of volatile products common to both model system and dehydrated carrots comprised 40 to 50% of the total volatiles from carrots.

11. The mathematical model correlating beta-carotene loss and production of dihydroactinidiolide as a function of temperature was satisfactory for the monitoring beta-carotene loss in dehydrated carrot.

RECOMMENDATIONS FOR FURTHER RESEARCH

1. Previous kinetic models have not been related adequately to observed products. This research could be extended by doing detailed mechanism studies, including electron spin resonance to observe the free radicals involved in the process.

2. Volatile degradation products of beta-carotene have been associated with off-flavor and food acceptability. Future research could determine which volatile products have an impact on food unacceptability as well as on the level of volatile products necessary to affect product acceptability.

3. The stability of beta-carotene has been associated with water activity, presence of pro- and antioxidants and presence of lipids. The effect of these factors on the loss of beta-carotene should be investigated so that a kinetic model can be describe the process.

APPENDICES

APPENDIX I

DATA FOR CHAPTER I

KINETICS OF BETA-CAROTENE OXIDATION. PART III. DEVELOPMENT OF METHODOLOGY.

TABLE 1:

Contribution of non-volatile compounds separated by HPLC to the absorbance at 450 nm at the time of maximum absorbance. Figure 5.

TABLE 2:

Beta-carotene concentration by normal phase HPLC during autoxidation at 20 and 80°C, photosensitized and metachloroperbenzoic acid oxidation. Figure 6.

TABLE 3:

Relative concentration of volatiles at 50% loss of beta-carotene during autoxidation at 20 and 80°C, photosensitized and metachloroperbenzoic acid oxidation.

TABLE 1. CONTRIBUTION OF NON VOLATILE COMPOUNDS
SEPARATED BY HPLC
TO THE ABSORBANCE AT 450 NM AT
THE TIME OF MAXIMUM ABSORBANCE

RETENTION TIME (min.)	RELATIVE AREA			
	20 C	80 C	PHOTO- SENSIT.	CHEMICAL
3:00	100	100	100	100
4:00	14.9	37.5	18.57	3.83
6:30	9.62			
7:12	10.82	33.93	40.84	68.52
11:00			6.8	7.4
11:50			6.2	2.7
13:05				2.76

TABLE 2. BETA-CAROTENE CONCENTRATION

TIME, hrs	% BETA- CAROTENE			
	20 C	80 C	PHOTO- SENSIT.	CHEMICAL
0		100	100	100
0		100	100	100
0		100	100	100
1		66.59		
1		65		
1		66.62		
3		53.1		
3		53.41		
3		55.49		
6		39.96	52.82	54.82
6		38.04	59.94	69.74
6		40.05	62.22	55.73
9	90.68			
9	92			
9	94.12			
10		32.23		
10		27.12		
10		28.99		
11				28.05
11				37.25
11				30.21
12			58.3	
12			53.12	
12			55.82	
16				27.15
16				36.04
16				29.24
21			52.06	
21			52.23	
21			42	
30	75.56			18.18
30	76.37			20.58
30	79.14			16.94
33			41.76	
33			32.98	
33			32.64	
40				11.79
40				15.68
40				14.7
50	63.52			
50	63.95			
50	67.1			
71			31.19	
71			31.78	
71			23.72	
82	48.11			
82	48.15			
82	51.52			

TABLE 3. NORMALIZED AREA OF VOLATILES AT
50% BETA-CAROTENE LOSS
DURING OXIDATION.

RELATIVE AREA						
PEAK #	CONTROL	20 C	80 C	PHOTO- SENSIT.	CHEMICAL	
11	0	6.229203	0	0	0	0
	0	0	0	0	0	4.928640
	0	31.83219	0	4.549966	5.457601	
14	67.46035	99.99998	.5599349	14.86356	34.93439	
16	0	10.85820	0	0	0	0
17	100.0000	16.06990	.1633524	9.434120	47.81590	
	0	6.302723	.9065717	0	0	0
	0	2.469332	0	.1636386	0	0
	19.66324	0	.5441676	0	0	0
18	0	0	0	7.156164	10.24622	
	14.14606	8.537080	0	.7512916	14.06671	
19	0	0	.2420079	9.172018	8.409820	
	0	0	2.095929	0	0	0
	0	4.934083	.2700541	2.541020	26.52275	
	0	0	0	2.012905	0	0
21	15.24292	13.19661	7.056999	100.0000	100.0000	
22	0	81.79343	52.67378	52.97874	38.86318	
	0	11.31683	.6374011	10.44751	7.380725	
	0	17.24924	6.803997	10.71384	19.23721	
	0	2.617464	4.658765	2.377635	0	0
	0	0	1.976024	0	0	0
	0	3.487395	5.237925	1.314467	0	0
	0	0	1.910589	1.640809	0	0
	0	7.275952	7.042927	3.986683	0	0
23	0	44.94672	100.0000	45.78487	65.03991	
	0	0	1.419557	0	0	0
	0	0	1.159188	2.904022	0	0

APPENDIX II

DATA FOR CHAPTER II

KINETICS OF BETA-CAROTENE OXIDATION. PART II.
BETA-CAROTENE DEGRADATION AND VOLATILE FORMATION
IN A MODEL SYSTEM.

TABLES 1 to 4:

First order kinetics for the loss of beta-carotene at 25, 40, 60 and 80°C.

TABLE 5:

Linear regression analysis for $\ln C/C_0$ vs. time at 25, 40, 60 and 80°C.

TABLES 6 TO 9:

Free radical recombination kinetics for the loss of beta-carotene at 25, 40, 60 and 80°C.

TABLE 10:

Linear regression analysis for $\ln \nabla$ vs time at 25, 40, 60 and 80°C.

TABLES 11 TO 14:

Kinetics of beta-carotene loss by the 1.5 order reaction at 25, 40, 60 and 80°C.

TABLE 15:

Linear regression analysis for $\ln k_{1.5}$ vs time at 25, 40, 60 and 80°C.

TABLE 16:

Arrhenius plot for the loss of beta-carotene according to first order reaction, free radical recombination and 1.5 order reaction.

TABLES 17 TO 20:

Production of volatile degradation products during oxidation of beta-carotene at 25, 40, 60 and 80°C.

TABLES 21 TO 24:

Linear regression for $\text{Bond}_1/\text{Bond}_2$ at 25, 40, 60 and 80°C up to 50% beta-carotene loss.

TABLE 25:

Rate of production of volatile degradation products from cleavage at bonds 1, 2, 3 and 4 during oxidation of beta-carotene at 25, 40, 60 and 80°C.

TABLE 26:

Arrhenius relationship for the production of volatile products during oxidation of beta-carotene.

TABLE 1. CONCENTRATION OF BETA-CAROTENE IN AVICEL.
AUTOXIDATION AT 25 C

	TIME (HRS)	C mg/100g	C/Co	ln C/Co
REPLICATE	0	100	1	0
No. 1	30	73.773	.73773	-.30418
	79	47.178	.47178	-.75125
	108	44.64	.4464	-.80653
	136	16.388	.16388	-1.8086
	221	6.213	.06213	-2.7786
REPLICATE	0	100	1	0
No. 2	30	73.773	.73773	-.30418
	79	50.989	.50989	-.67356
	108	40.676	.40676	-.89952
	136	17.667	.17667	-1.7335
	221	6.396	.06396	-2.7494
REPLICATE	0	100	1	0
No. 3	30	73.74	.7374	-.30462
	79	53.561	.53561	-.62434
	108	39.838	.39838	-.92034
	136	19.472	.19472	-1.6362
	221	6.397	.06397	-2.7494
REPLICATE	0	100	1	0
No. 4	30	73.418	.73418	-.309
	79	51.253	.51253	-.6684
	108	42.391	.42391	-.85823
	136	18.203	.18203	-1.7036
	221	6.44	.0644	-2.8061
REPLICATE	0	100	1	0
No. 5	30	73.096	.73096	-.3134
	79	49.654	.49654	-.7001
	108	41.201	.41201	-.8867
	136	17.435	.17435	-1.7467
	221	5.752	.05752	-2.8556
AVERAGE	0	100	1	0
	30	73.56	.7356	-.307076
	79	50.527	.50527	-.68353
	108	41.7492	.417492	-.874264
	136	17.833	.17833	-1.72572
	221	6.2396	.062396	-2.78782

TABLE 2. CONCENTRATION OF BETA-CAROTENE IN AVICEL.
AUTOXIDATION AT 40 C

	TIME (HRS)	C mg/100g	C/Co	ln C/Co
REPLICATE	0	100	1	0
No. 1	10	69.406	.69406	-.36519
	30	51.857	.51857	-.65668
	43	33.062	.33062	-1.1068
	58	23.977	.23977	-1.42806
	72	17.902	.17902	-1.72024
	86	11.803	.11803	-2.13684
REPLICATE	0	100	1	0
No. 2	10	75.55	.7555	-.28038
	30	47.465	.47465	-.74518
	43	39.277	.39277	-.93454
	58	24.133	.24133	-1.4216
	72	18.86	.1886	-1.66812
	86	12.356	.12356	-2.09105
REPLICATE	0	100	1	0
No. 3	10	92.192	.92192	-.0813
	30	69.877	.69877	-.35843
	43	45.284	.45284	-.79222
	58	23.273	.23273	-1.45787
	72	16.571	.16571	-1.79752
	86	11.275	.11275	-2.18263
REPLICATE	0	100	1	0
No. 4	10	81.709	.81709	-.202
	30	58.878	.58878	-.5297
	43	40.398	.40398	-.9064
	58	23.876	.23876	-1.4323
	72	18.023	.18023	-1.7135
	86	11.939	.11939	-2.1254
REPLICATE	0	100	1	0
No. 5	10	76.109	.76109	-.273
	30	53.451	.53451	-.6264
	43	37.34	.3734	-.9851
	58	23.45	.2345	-1.4504
	72	17.449	.17449	-1.7459
	86	11.668	.11668	-2.1483
AVERAGE	0	100	1	0
	10	78.9932	.789932	-.240374
	30	56.3056	.563056	-.583278
	43	39.0722	.390722	-.945012
	58	23.7418	.237418	-1.43805
	72	17.761	.17761	-1.72906
	86	11.8082	.118082	-2.13684

TABLE 3. CONCENTRATION OF BETA-CAROTENE IN AVICEL.
AUTOXIDATION AT 60 C

	TIME (HRS)	C mg/100g	C/Co	ln C/Co
REPLICATE No. 1	0	100	1	0
	3	66.279	.66279	-.4113
	10	25.615	.25615	-.25615
	15	14.18	.1418	-1.953
	20	7.644	.07644	-2.57126
	25	6.38	.0638	-2.75206
	30	2.178	.02178	-3.82699
REPLICATE No. 2	0	100	1	0
	3	77.476	.77476	-.2552
	10	41.206	.41206	-.88659
	15	18.159	.18159	-1.706
	20	8.6104	.086104	-2.41795
	25	8.542	.08542	-2.46015
	30	2.315	.02315	-3.76592
REPLICATE No. 3	0	100	1	0
	3	81.01	.8101	-.2106
	10	48.47	.4847	-.72422
	15	26.762	.26762	-1.3182
	20	15.782	.15782	-1.84628
	25	9.016	.09016	-2.40614
	30	3.486	.03486	-3.35651
REPLICATE No. 4	0	100	1	0
	3	76.193	.76193	-.2719
	10	39.681	.39681	-.9243
	15	20.724	.20724	-1.5739
	20	11.413	.11413	-2.1704
	25	10.633	.10633	-2.2412
	30	2.797	.02797	-3.5765
REPLICATE No. 5	0	100	1	0
	3	72.464	.72464	-.32208
	10	33.834	.33834	-1.0837
	15	17.67	.1767	-1.73328
	20	9.521	.09521	-2.3517
	25	7.003	.07003	-2.6589
	30	2.487	.02487	-3.6941
AVERAGE	0	100	1	0
	3	74.6844	.746844	-.294216
	10	37.7612	.377612	-.774992
	15	19.499	.19499	-1.65688
	20	10.59408	.1059408	-2.27152
	25	8.3148	.083148	-2.50369
	30	2.6526	.026526	-3.64400

TABLE 4. CONCENTRATION OF BETA-CAROTENE IN AVICEL.
AUTOXIDATION AT 80 C

	TIME (HRS)	C mg/100g	C/Co	ln C/Co
REPLICATE	0	100	1	0
No. 1	1	80.977	.80977	-.211
	3	53.104	.53104	-.63292
	6	39.958	.39958	-.91733
	10	32.234	.32234	-1.13216
	16	3.922	.03922	-3.23857
	30	1.262	.01262	-4.37277
REPLICATE	0	100	1	0
No. 2	1	81.131	.81131	-.2091
	3	53.41	.5341	-.62718
	6	39.036	.39036	-.94069
	10	27.123	.27123	-1.30478
	16	3.122	.03122	-3.4666
	30	.917	.00917	-4.69198
REPLICATE	0	100	1	0
No. 3	1	82.177	.82177	-.1963
	3	55.485	.55485	-.58905
	6	40.05	.4005	-.91504
	10	28.985	.28985	-1.23839
	16	2.998	.02998	-3.5071
	30	.999	.00999	-4.60661
REPLICATE	0	100	1	0
No. 4	1	81.612	.81612	-.2032
	3	54.357	.54357	-.6096
	6	39.772	.39772	-.922
	10	30.062	.30062	-1.2019
	16	3.465	.03465	-3.3626
	30	1.099	.01099	-4.511
REPLICATE	0	100	1	0
No. 5	1	81.31	.8131	-.2069
	3	53.768	.53768	-.6205
	6	39.558	.39558	-.9274
	10	28.794	.28794	-1.245
	16	3.24	.0324	-3.4297
	30	1.0145	.010145	-4.5908
AVERAGE	0	100	1	0
	1	81.4414	.814414	-.2053
	3	54.0248	.540248	-.61585
	6	39.6748	.396748	-.924492
	10	29.4396	.294396	-1.22445
	16	3.3494	.033494	-3.40091
	30	1.0583	.010583	-4.55463

TABLE 5. LINEAR REGRESSION ANALYSIS.
BETA-CAROTENE AUTOXIDATION
FIRST ORDER REACTION.

	25 C	40 C	60 C	80 C
REP. No. 1				
slope	-.00947	-.0208	-.13613	-.21097
interc	-.00767	-.0627	-.001199	-.00001
correl	.9992	.9365	1	1
REP. No. 2				
slope	-.00843	-.0246	-.08893	-.20906
interc	-.0196	-.0137	.004739	-.00002
correl	.9933	.9977	.9998	1
REP. No. 3				
slope	-.00777	-.0122	-.072591	-.19635
interc	-.0274	.0164	.002953	.00002
correl	.98487	.9867	.9999	1
REP. No. 4				
slope	-.00835	-.01748	-.09257	-.2032
interc	-.02238	-.0109	.002388	0
correl	.9911	.9971	1	.9999
REP. No. 5				
slope	-.008767	-.02042	-.10845	-.20683
interc	-.01929	-.02751	.001342	-.00003
correl	.994	.9866	1	1
RATE CONSTANT				
AVERAGE	.00856	.0191	.0998	.2053

TABLE 6. FREE RADICAL RECOMBINATION KINETICS
BETA-CAROTENE AUTOXIDATION AT 25 C

	TIME (HRS)	$\ln \frac{(1 + \sqrt{1 - C/C_0})}{(1 - \sqrt{1 - C/C_0})}$
REPLICATE	0	0
No. 1	30	.9506037
	79	1.721609
	108	1.803321
	136	3.065678
	221	4.117356
REPLICATE	0	0
No. 2	30	.9506037
	79	1.603085
	108	1.936406
	136	2.979864
	221	4.086901
REPLICATE	0	0
No. 3	30	.9515255
	79	1.525451
	108	1.965526
	136	2.867379
	221	4.086737
REPLICATE	0	0
No. 4	30	.9605176
	79	1.595036
	108	1.877930
	136	2.945480
	221	4.079703
REPLICATE	0	0
No. 5	30	.9695058
	79	1.644090
	108	1.918350
	136	2.995024
	221	4.198035
AVERAGE	0	0
	30	.9565513
	79	1.617854
	108	1.900307
	136	2.970685
	221	4.113746

TABLE 7. FREE RADICAL RECOMBINATION KINETICS
BETA-CAROTENE AUTOXIDATION AT 40 C

	TIME (HRS)	$\ln \frac{(1 + \sqrt{1 - C/C_0})}{(1 - \sqrt{1 - C/C_0})}$
REPLICATE	0	0
No. 1	10	1.245726
	30	1.710690
	43	2.302432
	58	2.681996
	72	3.010355
	86	3.461298
REPLICATE	0	0
No. 2	10	1.083918
	30	1.835412
	43	2.086915
	58	2.674554
	72	2.952653
	86	3.412466
REPLICATE	0	0
No. 3	10	.5741230
	30	1.233452
	43	1.899645
	58	2.716095
	72	3.095274
	86	3.509957
REPLICATE	0	0
No. 4	10	.9141072
	30	1.520636
	43	2.050634
	58	2.686835
	72	3.002917
	86	3.449094
REPLICATE	0	0
No. 5	10	1.068923
	30	1.666691
	43	2.151302
	58	2.707441
	72	3.038602
	86	3.473543
AVERAGE	0	0
	10	.9773596
	30	1.593376
	43	2.098185
	58	2.693384
	72	3.019960
	86	3.461271

TABLE 8. FREE RADICAL RECOMBINATION KINETICS
BETA-CAROTENE AUTOXIDATION AT 60 C

	TIME (HRS)	$\ln \frac{(1 + \sqrt{1 - C/C_0})}{(1 - \sqrt{1 - C/C_0})}$
REPLICATE No. 1	0	0
	3	1.327030
	10	2.605796
	15	3.264634
	20	3.918179
	25	4.105605
	30	5.202077
REPLICATE No. 2	0	0
	3	1.031968
	10	2.024895
	15	2.994611
	20	3.793981
	25	3.802322
	30	5.140378
REPLICATE No. 3	0	0
	3	.9340074
	10	1.806365
	15	2.554810
	20	3.148557
	25	3.745778
	30	4.725047
REPLICATE No. 4	0	0
	3	1.066665
	10	2.073760
	15	2.847424
	20	3.497037
	25	3.572083
	30	4.948783
REPLICATE No. 5	0	0
	3	1.165738
	10	2.274138
	15	3.024741
	20	3.688564
	25	4.009154
	30	5.067835
AVERAGE	0	0
	3	1.105082
	10	2.156991
	15	2.937244
	20	3.609264
	25	3.846988
	30	5.016824

TABLE 9. FREE RADICAL RECOMBINATION KINETICS
BETA-CAROTENE AUTOXIDATION AT 80 C

	TIME (HRS)	$\ln \frac{(1 + \sqrt{1-C/C_0})}{(1 - \sqrt{1-C/C_0})}$
REPLICATE	0	0
No. 1	1	.9349419
	3	1.676219
	6	2.064793
	10	2.333336
	16	4.604958
	30	5.752427
REPLICATE	0	0
No. 2	1	.9305769
	3	1.667815
	6	2.094805
	10	2.539134
	16	4.837195
	30	6.073512
REPLICATE	0	0
No. 3	1	.9006666
	3	1.611343
	6	2.061824
	10	2.460860
	16	4.878358
	30	5.987451
REPLICATE	0	0
No. 4	1	.9168810
	3	1.641935
	6	2.070810
	10	2.417402
	16	4.731197
	30	5.891546
REPLICATE	0	0
No. 5	1	.9254912
	3	1.658009
	6	2.077756
	10	2.468700
	16	4.799491
	30	5.971977
AVERAGE	0	0
	1	.9217115
	3	1.651064
	6	2.073998
	10	2.443886
	16	4.770240
	30	5.935382

TABLE 10. LINEAR REGRESSION ANALYSIS
BETA-CAROTENE AUTOXIDATION
FREE RADICAL RECOMBINATION.

	25 C	40 C	60 C	80 C
REP. No. 1				
slope	.021201	.052198	.24677	.53187
interc	.12042	.2895	.24158	.16123
correl	.9613	.8125	.9446	.9355
REP. No. 2				
slope	.019612	.057808	.19174	.52918
interc	.13868	.20233	.18807	.16056
correl	.9412	.9159	.9444	.9354
REP. No. 3				
slope	.018568	.039969	.17071	.51115
interc	.15101	.06977	.173714	.15558
correl	.9237	.9777	.9404	.9349
REP. No. 4				
slope	.019484	.047779	.19612	.52091
interc	.143938	.174527	.19695	.15839
correl	.9362	.909	.9418	.9352
REP. No. 5				
slope	.020124	.05189	.21517	.52604
interc	.14003	.22001	.214207	.15978
correl	.943	.8812	.9428	.9353
RATE CONSTANT				
AVERAGE	.0198	.0499	.2041	.5238

TABLE 11. 1.5 ORDER REACTION
BETA-CAROTENE AUTOXIDATION - 25 C

	TIME (hrs)	C mg/100g	$\sqrt{C_0} - \sqrt{C}$
REPLICATE	0	100	0
No. 1	30	73.773	1.410879
	79	47.178	3.131376
	108	44.64	3.318683
	136	16.388	5.951791
	221	6.213	7.507411
REPLICATE	0	100	0
No. 2	30	73.773	1.410879
	79	50.989	2.859342
	108	40.676	3.622226
	136	17.667	5.796787
	221	6.396	7.470969
REPLICATE	0	100	0
No. 3	30	73.74	1.412800
	79	53.561	2.681462
	108	39.838	3.688265
	136	19.472	5.587291
	221	6.397	7.470771
REPLICATE	0	100	0
No. 4	30	73.418	1.431570
	79	51.253	2.840880
	108	42.391	3.489163
	136	18.203	5.733503
	221	6.44	7.462284
REPLICATE	0	100	0
No. 5	30	73.096	1.450380
	79	49.654	2.953441
	108	41.201	3.581199
	136	17.435	5.824476
	221	5.752	7.601667
AVERAGE	0	100	0
	30	73.56	1.423287
	79	50.527	2.891765
	108	41.7492	3.538638
	136	17.833	5.777086
	221	6.2396	7.502081

TABLE 12. 1.5 ORDER REACTION
BETA-CAROTENE AUTOXIDATION - 40 C

REPLICATE	TIME (hrs)	C mg/100g	$\sqrt{C_0} - \sqrt{C}$
No. 1	0	100	0
	10	69.406	1.668974
	30	51.857	2.798820
	43	33.062	4.250043
	58	23.977	5.103369
	72	17.902	5.768924
	86	11.803	6.564451
No. 2	0	100	0
	10	75.55	1.308050
	30	47.465	3.110515
	43	39.277	3.732863
	58	24.133	5.087465
	72	18.86	5.657190
	86	12.356	6.484890
No. 3	0	100	0
	10	92.192	.3983335
	30	69.877	1.640754
	43	45.284	3.270661
	58	23.273	5.175790
	72	16.571	5.929251
	86	11.275	6.642173
No. 4	0	100	0
	10	81.709	.9606969
	30	58.878	2.326800
	43	40.398	3.644058
	58	23.876	5.113693
	72	18.023	5.754650
	86	11.939	6.544714
No. 5	0	100	0
	10	76.109	1.275953
	30	53.451	2.688981
	43	37.34	3.889354
	58	23.45	5.157480
	72	17.449	5.822800
	86	11.668	6.584155
AVERAGE	0	100	0
	10	78.9932	1.112188
	30	56.3056	2.496294
	43	39.0722	3.749224
	58	23.7418	5.127444
	72	17.761	5.785620
	86	11.8082	6.563694

TABLE 13. 1.5 ORDER REACTION
BETA-CAROTENE AUTOXIDATION - 60 C

REPLICATE	TIME (hrs)	C mg/100g	$\sqrt{C_0} - \sqrt{C}$
No. 1	0	100	0
	3	66.279	1.858808
	10	25.615	4.938874
	15	14.18	6.234366
	20	7.644	7.235222
	25	6.38	7.474134
	30	2.178	8.524195
No. 2	0	100	0
	3	77.476	1.197955
	10	41.206	3.580810
	15	18.159	5.738662
	20	8.6104	7.065652
	25	8.542	7.077330
	30	2.315	8.478488
No. 3	0	100	0
	3	81.01	.9994445
	10	48.47	3.037960
	15	26.762	4.826800
	20	15.782	6.027343
	25	9.016	6.997335
	30	3.486	8.132917
No. 4	0	100	0
	3	76.193	1.271140
	10	39.681	3.700714
	15	20.724	5.447638
	20	11.413	6.621687
	25	10.633	6.739172
	30	2.797	8.327577
No. 5	0	100	0
	3	72.464	1.487421
	10	33.834	4.183300
	15	17.67	5.796430
	20	9.521	6.914388
	25	7.003	7.353682
	30	2.487	8.422977

TABLE 14. 1.5 ORDER REACTION
BETA-CAROTENE AUTOXIDATION - 80 C

REPLICATE	TIME (hrs)	C mg/100g	$\sqrt{C_0} - \sqrt{C}$
No. 1	0	100	0
	1	80.977	1.001278
	3	53.104	2.712751
	6	39.958	3.678766
	10	32.234	4.322501
	16	3.922	8.019596
	30	1.262	8.876612
No. 2	0	100	0
	1	81.131	.9927252
	3	53.41	2.691785
	6	39.036	3.752120
	10	27.123	4.792025
	16	3.122	8.233082
	30	.917	9.042399
No. 3	0	100	0
	1	82.177	.9348469
	3	55.485	2.551175
	6	40.05	3.671493
	10	28.985	4.616228
	16	2.998	8.268527
	30	.999	9.000500
No. 4	0	100	0
	1	81.612	.9660640
	3	54.357	2.627280
	6	39.772	3.693495
	10	30.062	4.517118
	16	3.465	8.138549
	30	1.099	8.951668
No. 5	0	100	0
	1	81.31	.9827942
	3	53.768	2.667333
	6	39.558	3.710485
	10	28.794	4.633996
	16	3.24	8.2
	30	1.0145	8.992776

TABLE 15. LINEAR REGRESSION ANALYSIS
BETA-CAROTENE AUTOXIDATION.
1.5 ORDER REACTION

	25 C	40 C	60 C	80 C
REP. No. 1				
slope	.03449	.07554	.2719	.2932
interc	.2539	.367	1.1799	1.323
correl	.9565	.9515	.9206	.8754
REP. No. 2				
slope	.03441	.07355	.2831	.3006
interc	.235	.4843	.5685	1.3807
correl	.9709	.9801	.9579	.8716
REP. No. 3				
slope	.03425	.08425	.2737	.302
interc	.1965	-.3049	.2611	1.3017
correl	.9789	.9788	.9911	.8724
REP. No. 4				
slope	.03405	.07806	.2701	.2979
interc	.2206	.1436	.6119	1.3186
correl	.9704	.9903	.9632	.8747
REP. No. 5				
slope	.03482	.07632	.2764	.2992
interc	.2375	.3714	.8134	1.3485
correl	.9719	.9868	.9549	.8727
RATE CONSTANT				
AVERAGE	.034404	.077544	.27504	.29858

TABLE 16. ARRHENIUS PLOT
BETA-CAROTENE AUTOXIDATION

	1/T K	LN RATE CONSTANT		
		1ST ORDER	RADICAL RECOMB.	EMPIRICAL MODEL
REPLICATE	.0033557	-4.65963	-3.85371	-3.3671
No. 1	.0031949	-3.8728	-2.95271	-2.5831
	.003003	-1.99415	-1.3993	-1.3023
	.002833	-1.55604	-.63136	-1.2269
REPLICATE	.0033557	-4.77596	-3.93161	-3.3694
No. 2	.0031949	-3.70501	-2.85063	-2.6098
	.003003	-2.41991	-1.65162	-1.262
	.002833	-1.56513	-.63643	-1.202
REPLICATE	.0033557	-4.85749	-3.98632	-3.3741
No. 3	.0031949	-4.40632	-3.21965	-2.474
	.003003	-2.62291	-1.76779	-1.2957
	.002833	-1.62786	-.67109	-1.1973
REPLICATE	.0033557	-4.78549	-3.93816	-3.3799
No. 4	.0031949	-4.0467	-3.04117	-2.5503
	.003003	-2.37979	-1.62903	-1.309
	.002833	-1.59356	-.65218	-1.211
REPLICATE	.0033557	-4.73676	-3.90584	-3.3576
No. 5	.0031949	-3.89124	-2.95863	-2.5728
	.003003	-2.22147	-1.53633	-1.2859
	.002833	-1.57586	-.64238	-1.2066
AVERAGE	.0033557	-4.76307	-3.92313	-3.36962
	.0031949	-3.98441	-3.00456	-2.558
	.003003	-2.32765	-1.59681	-1.29098
	.002833	-1.58369	-.646688	-1.20876

TABLE 17. GC RESPONSE FOR ALL VOLATILE PRODUCTS
FROM BONDS 1 TO 4
AUTOXIDATION OF BETA-CAROTENE - 25 C

	TIME, HRS	BOND 1	BOND 2	BOND 3	BOND 4
REPLICATE	0	0	0	0	0
No. 1	10	293959	572596	22706	231060
	31	630757	847434	85441	460455
	79	1049650	1195776	288111	948416
	108	1478731	1472077	333897	1262218
	153	1825519	1654315	1069599	2446209
	221	2364713	1960185	1868752	3691816
REPLICATE	0	0	0	0	0
No. 2	10	297192	569483	20591	216735
	31	631797	834210	81942	445297
	79	1071900	1215102	288885	938859
	108	1618658	1585772	347181	1312551
	153	1981355	1771337	1142651	2556188
	221	2016351	1752939	1685166	3426921
REPLICATE	0	0	0	0	0
No. 3	10	290713	575708	24821	245386
	31	629716	860649	88940	475612
	79	1027400	1176449	287338	957972
	108	1338805	1358382	320614	1211885
	153	1669684	1537294	996547	2336230
	221	2190532	1856562	1776959	3559369
REPLICATE	0	0	0	0	0
No. 4	10	265808	517812	15221	199618
	31	620098	812426	87160	455063
	79	1065329	1175287	295504	954683
	108	1494455	1481098	344260	1302033
	153	1843580	1663029	1061563	2491519
	221	2187841	1865826	1763998	3602251
REPLICATE	0	0	0	0	0
No. 5	10	322098	627380	30191	262503
	31	641415	882443	83723	465846
	79	1033971	1216264	280719	942148
	108	1463007	1463056	323534	1222404
	153	1807459	1645602	1077634	2400898
	221	2193223	1847297	1789920	3516487
AVERAGE	0	0	0	0	0
	10	293954	572595.8	22706	231060.4
	31	630756.6	847432.4	85441.2	460454.6
	79	1049650	1195776.	288111.4	948415.6
	108	1478731.	1472077	333897.2	1262218.
	153	1825519.	1654315.	1069599.	2446209.
	221	2190532	1856562.	1776959	3559369.

TABLE 18.GC RESPONSE FOR ALL VOLATILE PRODUCTS
FROM BONDS 1 TO 4.
AUTOXIDATION OF BETA-CAROTENE - 40 C

	TIME, HRS	BOND 1	BOND 2	BOND 3	BOND 4
REPLICATE	0	0	0	0	0
No. 1	10	187424	168323	77469	316834
	29	444338	269150	131753	470465
	43	720294	355921	306198	938687
	58	900750	401540	454472	1248519
	72	1014017	423214	645369	1679048
	86	1090837	435710	840399	2040520
REPLICATE	0	0	0	0	0
No. 2	10	225286	206416	44894	257510
	29	431681	271520	51840	280312
	43	755779	373594	123303	475434
	58	973996	426882	148033	539621
	72	1104112	447390	222743	795550
	86	1179841	454390	363948	1112104
REPLICATE	0	0	0	0	0
No. 3	10	193961	144213	110792	404008
	29	480427	295453	192218	622938
	43	736591	379800	431554	1254776
	58	968108	448229	647104	1702545
	72	1115340	480739	933449	2341240
	86	1187893	499484	1440744	2695061
REPLICATE	0	0	0	0	0
No. 4	10	143025	154342	76722	288986
	29	420906	240478	151202	508147
	43	668512	314371	363739	1085852
	58	760148	329511	568279	1503392
	72	822601	341513	779914	1900356
	86	904779	353258	1016504	2314397
REPLICATE	0	0	0	0	0
No. 5	10	166541	126855	121401	414499
	29	475835	285106	225339	698883
	43	707501	360059	511699	1458248
	58	896825	415772	787186	2023802
	72	1021502	445446	1119173	2709508
	86	1096206	465773	1358263	3095825
AVERAGE	0	0	0	0	0
	10	183247.4	160029.8	86255.6	336367.4
	29	450637.4	272341.4	150470.4	516149
	43	717735.4	356749	347298.6	1042599.
	58	899965.4	404386.8	521014.8	1403576.
	72	1015514.	427660.4	740129.6	1885140.
	86	1091911.	441723	1003972.	2251581.

TABLE 19. GC RESPONSE FOR ALL VOLATILE PRODUCTS
FROM BONDS 1 TO 4.
AUTOXIDATION OF BETA-CAROTENE - 60 C

	TIME, HRS	BOND 1	BOND 2	BOND 3	BOND 4
REPLICATE	0	0	0	0	0
No. 1	10	1603020	1087310	2710333	7908614
	20	1871075	1243478	7606367	15428104
	30	2007864	1283032	9933500	16997832
	40	2134818	1338480	11404833	17858222
	53	2253622	1367847	12798167	18605861
REPLICATE	0	0	0	0	0
No. 2	10	2077973	1418383	1754800	5202538
	20	2359091	1567834	7975300	14756253
	30	2494140	1608632	9629800	15915296
	40	2601080	1647225	10848550	16659762
	53	2730381	1677648	12160550	17415579
REPLICATE	0	0	0	0	0
No. 3	10	512662	419570	3895100	10518878
	20	665974	516208	7254350	14092784
	30	831047	575540	10008800	15970744
	40	996115	650872	11406300	16818784
	53	1084781	664500	12895350	17547606
REPLICATE	0	0	0	0	0
No. 4	10	2218425	1423977	2481100	8004426
	20	2588160	1646392	7589450	17435275
	30	2698407	1664926	10161900	19107457
	40	2807259	1144896	11959650	20096121
	53	2945703	1761395	13338600	20854399
REPLICATE	0	0	0	0	0
No. 5	10	559479	421434	4137200	11452841
	20	742330	542393	7125733	14985791
	30	899136	594304	10186167	17034797
	40	664077	674245	11776667	17964236
	53	1156555	692415	13288033	18693879
AVERAGE	0	0	0	0	0
	10	1394312.	954134.8	2995707.	8617459.
	20	1645326	1103261	7510240	15339641
	30	1786119.	1145287.	9984033.	17005225
	40	1840670.	1091144.	11479200	17879425
	53	2034208.	1232761	12896140	18623465

TABLE 20. GC RESPONSE FOR ALL VOLATILE PRODUCTS
FROM BONDS 1 TO 4.
AUTOXIDATION OF BETA-CAROTENE - 80 C

	TIME, HRS	BOND 1	BOND 2	BOND 3	BOND 4
REPLICATE	0	0	0	0	0
No. 1	1	531253	731700	732630	1669268
	6	6130379	3606735	8903930	14389419
	16	7168312	3783553	14828930	18746641
	28	7738426	3947264	17376230	20846963
	40	8365398	4166634	19117030	21837874
REPLICATE	0	0	0	0	0
No. 2	1	980722	695164	469220	1318087
	6	2506461	2740584	4817120	14887295
	16	2861586	2946501	8737120	18732470
	28	3215248	3100951	11116820	21483122
	40	3627532	3241485	12947120	23728802
REPLICATE	0	0	0	0	0
No. 3	1	755988	713432	600925	1493678
	6	4318571	3173660	6860525	14638358
	16	5014950	3365028	11783025	18739557
	28	5476838	3524109	14246525	21165044
	40	5996466	3704061	16032075	22783340
REPLICATE	0	0	0	0	0
No. 4	1	658282	722566	666778	1581473
	6	5239287	3390198	7882228	14513889
	16	6106293	3574291	13305978	18743100
	28	6622294	3735687	15811378	21006005
	40	7195594	3935348	17574553	22310609
REPLICATE	0	0	0	0	0
No. 5	1	868355	646764	535073	1405883
	6	3412516	2899588	5838823	14762827
	16	3938268	3098231	10260073	18736014
	28	4346043	3254997	12681673	21324084
	40	4811999	3415240	14489598	23256072
AVERAGE	0	0	0	0	0
	1	758920	701925.2	600925.2	1493678.
	6	4321443.	3162153	6860525.	14638358
	16	5017882.	3353521.	11783025	18739556
	28	5479770.	3512602.	14246525	21165044
	40	5999398.	3692554.	16032075	22783339

TABLE 21. LINEAR REGRESSION FOR B_i/B_3
 BETA-CAROTENE AUTOXIDATION - 25 C
 FIRST THREE POINTS (10, 31, 79 hrs)
 UP TO 50% LOSS OF BETA-CAROTENE

		B1/B3	B2/B3	B4/B3
REP. 1	slope	2.6556	2.1935	2.6304
	interc.	307350	582211	199211
	correl.	.9467	.9491	.9919
REP. 2	slope	2.6942	2.2626	2.6137
	interc.	315443	577723	192620
	correl.	.9502	.9605	.991
REP. 3	slope	2.6162	2.1233	2.6473
	interc.	299492	587057	205744
	correl.	.9429	.9351	.9927
REP. 4	slope	2.6926	2.2107	2.6278
	interc.	293296	541970	187934
	correl.	.9572	.9549	.992
REP. 5	slope	2.6118	2.173	2.633
	interc.	32226	622844	210474
	correl.	.9339	.9425	.9917

TABLE 22. LINEAR REGRESSION FOR B_1/B_3
 BETA-CAROTENE AUTOXIDATION - 40 C
 FIRST THREE POINTS (10, 29, 43 HRS)
 UP TO 61% LOSS OF BETA-CAROTENE

		B_1/B_3	B_2/B_3	B_4/B_3
REP. 1	slope	2.1468	.7412	2.7103
	interc.	81853	137117	109689
	correl.	.9269	.8902	.9999
REP. 2	slope	1.5509	.6479	2.6495
	interc.	90582	114515	111824
	correl.	.9074	.819	1
REP. 3	slope	1.6841	.5113	2.7635
	interc.	78681	135560	82634
	correl.	.9103	.904	.9997
REP. 4	slope	1.2631	.526	2.66945
	interc.	88540	106840	93356
	correl.	.8848	.7974	1

TABLE 23. LINEAR REGRESSION FOR B_1/B_3
 BETA-CAROTENE AUTOXIDATION - 60 C
 FIRST TWO POINTS (10, 20 hrs)
 UP TO 90% LOSS OF BETA-CAROTENE

		B_1/B_3	B_2/B_3	B_4/B_3
REP. 1	slope	.05475	.0319	1.5358
	interc.	1454631	1000859	3745995
	correl.	1	1	1
REP. 2	slope	.04519	.02403	1.5358
	interc.	1998669	1376223	2507440
	correl.	1	1	1
REP. 3	slope	.04564	.02877	1.0639
	interc.	334895	307517	6374881
	correl.	1	1	1
REP. 4	slope	.07238	.04354	1.8462
	interc.	2038847	1315951	3423910
	correl.	1	1	1
REP. 5	slope	.06118	.04047	1.1822
	interc.	306348	253983	6561973
	correl.	1	1	1

TABLE 24. LINEAR REGRESSION FOR B_1/B_3
 BETA-CAROTENE AUTOXIDATION - 80 C
 FIRST TWO POINTS (1, 6 hrs)
 UP TO 61% BETA-CAROTENE LOSS

		B_1/B_3	B_2/B_3	B_4/B_3
REP. 1	slope	.6872	.383	1.5914
	interc.	1330	21587	24086
	correl.	1	1	1
REP. 2	slope	.4528	.5297	3.1026
	interc.	36445	21187	-6533
	correl.	1	1	1
REP. 3	slope	.6048	.4341	2.1199
	interc.	18728	21590	10485
	correl.	1	1	1
REP. 4	slope	.6524	.4052	1.8211
	interc.	106704	21620	17548
	correl.	1	1	1
REP. 5	slope	.5421	.4675	2.5243
	interc.	27527	18877	2626
	correl.	1	1	1

TABLE 25. RATE OF FORMATION OF VOLATILE PRODUCTS
FROM CLEAVAGE AT BONDS 1, 2, 3 & 4
BETA-CAROTENE AUTOXIDATION

	25 C	40 C	60 C	80 C
BOND 1	.00269	.006172	.002209	.02915
BOND 2	.002308	.002164	.001342	.02138
BOND 3	.001014	.002906	.01005	.0488
BOND 4	.002668	.007859	.056104	.106

TABLE 26. ACTIVATION ENERGY AND FREQUENCY FACTOR
FOR VOLATILE FORMATION
BETA-CAROTENE AUTOXIDATION

	Ea (Kcal/mol)	Frequency
BOND 1	6.66 + 1.3	188.7
BOND 2	6.94 + .83	175.9
BOND 3	16.12 + .29	7.69 x 10
BOND 4	14.68 + .76	1.75 x 10

APPENDIX III

DATA FOR CHAPTER III

COMPARISON OF METHODS FOR MEASURING
ALPHA- AND BETA-CAROTENE
IN CARROT PRODUCTS.

TABLE 1:

Recovery of alpha- and beta-carotene during extraction by methods: I- AOAC (1980), II- column chromatography, Wallace (1983), III- HPLC, Hsieh and Karel (1983)

TABLE 2:

Recovery of alpha- and beta-carotene during column chromatography by methods: I- AOAC (1980), II- column chromatography, Wallace (1983), III- HPLC, Hsieh and Karel (1983)

TABLE 3:

Total recovery of alpha- and beta-carotene during column chromatography by methods: I- AOAC (1980), II- column chromatography, Wallace (1983), III- HPLC, Hsieh and Karel (1983)

TABLE 4:

Alpha- and beta-carotene content (mg/100g) in dehydrated carrots by methods: I- AOAC (1980), II- column chromatography, Wallace (1983), III- HPLC, Hsieh and Karel (1983).

TABLE 5:

Alpha- and beta-carotene content (mg/100g) in carrot puree by methods: I- AOAC (1980), II- column chromatography, Wallace (1983), III- HPLC, Hsieh and Karel (1983).

TABLE 1. RECOVERY OF ALPHA AND BETA-CAROTENE
 EXTRACTION I- AOAC (1980)
 II- WALLACE (1983)
 III- HSIEH & KAREL (1983)

PERCENT RECOVERY		
	ALPHA-CAROTENE	BETA-CAROTENE
METHOD I		
COLD	91.75	106.82
	81.8	100.24
	93.23	95.14
	84.02	101.74
	109.42	107.53
HOT	99.82	96.79
	90.12	97.05
	80.24	77.31
	68.9	69.75
	68.98	70.56
METHOD II	82.61	98.33
	69.76	84.51
	75.23	75.57
	88.64	84.09
	64.36	62.59
METHOD III	93.71	100.24
	101.25	95.14
	90.45	101.74
	102.4	107.53
	96.95	101.16

**TABLE 2. RECOVERY OF ALPHA AND BETA-CAROTENE
COLUMN CHROMATOGRAPHY**

I- AOAC (1980)

II- WALLACE (1983)

III- HSIEH & KAREL (1983)

PERCENT RECOVERY

	ALPHA-CAROTENE	BETA-CAROTENE
METHOD I		
	89.68	93.14
	78.02	75.73
	92.96	87.58
	88.13	81.38
	88.12	89.48
METHOD II		
	66.12	95.95
	76.3	92.31
	97.77	104.99
	78.69	90.12
	76.22	102.44

TABLE 3. RECOVERY OF ALPHA AND BETA-CAROTENE
TOTAL RECOVERY

I- AOAC (1980)

II- WALLACE (1983)

III- HSIEH & KAREL (1983)

PERCENT RECOVERY

	ALPHA-CAROTENE	BETA-CAROTENE
METHOD I		
COLD	99.97	114.91
	81.72	88.62
	95.42	82.47
	67.1	85.68
	77.76	62.51
HOT		
	72.68	72.17
	53.46	59.29
	62.45	66.75
	66.26	73.88
	69.48	82.28
METHOD II		
	54.62	55.27
	53.23	62.66
	73.55	61.36
	69.75	51.59
	49.06	52.58
METHOD III		
	93.71	100.24
	101.25	95.14
	90.45	101.74
	102.4	107.53
	96.95	101.16

**TABLE 4. ALPHA- AND BETA-CAROTENE CONTENT (mg/100g)
DEHYDRATED CARROTS**

METHODS: I- AOAC (1980)

II- WALLACE (1983)

III- HSIEH & KAREL (1983)

METHOD			
	I	II	III
SAMPLE # 1			
ALPHA		33.24	65.14
		32.25	68.57
		32.6	64.87
BETA		73.87	126.11
		74.18	125.07
		70.77	128.66
TOTAL	158.06	107.11	191.25
	168.27	106.43	193.64
	166.92	103.37	193.53
SAMPLE # 2			
ALPHA		25.7	38.88
		27.72	35.92
		28.85	30.22
BETA		57.01	96.42
		52.24	77.42
		56.29	68.17
TOTAL	110.69	82.71	135.3
	112.84	79.96	113.39
	115.9	85.14	98.39

**TABLE 5. ALPHA- AND BETA-CAROTENE CONTENT (mg/100g)
CARROT PUREE AND FRESH CARROTS
METHODS: I- AOAC (1980)
II- WALLACE (1983)
III- HSIEH & KAREL (1983)**

	METHOD		
	I	II	III
CARROT PUREE 1			
ALPHA		3.67	2.96
		4	2.84
		4.49	3.37
BETA		5.63	5.64
		6.2	5.58
		6.83	6.88
TOTAL	9.24	9.3	8.6
	8.27	10.2	8.42
	10.03	11.32	10.25
CARROT PUREE 2			
ALPHA		2.22	3.59
		2.19	1.88
		2.18	1.72
BETA		4.88	9.61
		4.83	4.05
		4.14	4.21
TOTAL	7.23	7.1	13.2
	6.41	7.02	5.93
	5.88	6.32	5.93
FRESH CARROTS			
ALPHA		2.43	2.58
		2.45	2.59
		2.51	2.76
BETA		4.42	5.52
		4.1	5.94
		4.44	5.69
TOTAL	5.29	6.85	8.1
	6.15	6.55	8.53
	5.07	6.95	8.45

APPENDIX IV

DATA FOR CHAPTER IV

KINETICS OF BETA-CAROTENE OXIDATION. PART III.
BETA-CAROTENE DEGRADATION AND VOLATILE FORMATION
IN DEHYDRATED CARROTS.

TABLES 1 to 3:

First order kinetics for the loss of beta-carotene
at 20, 40 and 60°C.

TABLES 4 TO 6:

First order kinetics for the loss of alpha-
carotene at 20, 40 and 60°C.

TABLE 7:

Linear regression analysis for $\ln C/C_0$ vs. time at
20, 40 and 60°C for alpha- and beta-carotene.

TABLES 8 TO 10:

Production of volatile degradation products during
oxidation of beta-carotene in dehydrated carrots at 20,
40 and 60°C.

TABLES 11:

Linear regression for $\text{Bond}_1/\text{Bond}_3$ at 20, 40 and
60°C up to 50% loss of beta-carotene.

TABLE 12:

Rate of production of volatile degradation
products from cleavage at bonds 1, 2, 3 and 4 during
oxidation of beta-carotene at 20, 40 and 60°C.

TABLE 13:

Arrhenius relationship for the loss of alpha- and
beta-carotene during oxidation of beta-carotene in
dehydrated carrots.

TABLE 14:

Arrhenius relationship for the production of
volatile products during oxidation of beta-carotene in
dehydrated carrots.

TABLE 1. BETA-CAROTENE LOSS
AUTOXIDATION - 20 C
DEHYDRATED CARROTS

	TIME, hrs	PERCENT LOSS		
		C	C/Co	ln C/Co
REP. 1	0	100	1	0
	45	100	1	0
	81	100	1	0
	123	94.91	.9491	-.052241
	221	81.47	.8147	-.204935
	303	72.16	.7216	-.326284
	373	55.96	.5596	-.580533
REP. 2	0	100	1	0
	45	100	1	0
	81	100	1	0
	123	88.69	.8869	-.120023
	221	79.98	.7998	-.223394
	303	64.64	.6464	-.436337
	373	54.64	.5464	-.604404
REP. 3	0	100	1	0
	45	100	1	0
	81	100	1	0
	123	90.54	.9054	-.099378
	221	80.5	.805	-.216913
	303	64.13	.6413	-.444258
	373	50.68	.5068	-.679639
AVERAGE	0	100	1	0
	45	100	1	0
	81	100	1	0
	123	91.38	.9138	-.090548
	221	80.65	.8065	-.215081
	303	66.97667	.6697667	-.402293
	373	53.76	.5376	-.621525

TABLE 2. BETA-CAROTENE LOSS
 AUTOXIDATION - 40 C
 DEHYDRATED CARROTS

	TIME, hrs	PERCENT LOSS		
		C	C/Co	ln C/Co
REP. 1	0	100	1	0
	7	97.55	.9755	-.024805
	23	88.2	.882	-.125563
	32	81.02	.8102	-.210474
	56	62.42	.6242	-.471284
	85	60.48	.6048	-.502857
	153	45.17	.4517	-.794737
REP. 2	0	100	1	0
	7	95.7	.957	-.043952
	23	86.07	.8607	-.150009
	32	72.98	.7298	-.314985
	56	61.37	.6137	-.488249
	85	59.67	.5967	-.516341
	153	42.27	.4227	-.861093
REP. 3	0	100	1	0
	7	89.38	.8938	-.112273
	23	83.61	.8361	-.179007
	32	68.19	.6819	-.382872
	56	59.63	.5963	-.517011
	85	49.06	.4906	-.712126
	153	39.48	.3948	-.929376
AVERAGE	0	100	1	0
	7	94.21	.9421	-.060343
	23	85.96	.8596	-.151527
	32	74.06333	.7406333	-.302777
	56	61.14	.6114	-.492182
	85	56.40333	.5640333	-.577108
	153	42.30667	.4230667	-.861735

TABLE 3. BETA-CAROTENE LOSS
 AUTOXIDATION - 60 C
 DEHYDRATED CARROTS

	TIME, hrs	PERCENT LOSS		
		C	C/Co	ln C/Co
REP. 1	0	100	1	0
	22	72.1	.721	-.327116
	49	51.89	.5189	-.656044
	94	42.01	.4201	-.867263
	166	32.71	.3271	-1.11749
REP. 2	0	100	1	0
	22	57.18	.5718	-.558966
	49	48.58	.4858	-.721958
	94	37.21	.3721	-.988593
	166	31.96	.3196	-1.14069
REP. 3	0	100	1	0
	22	56.41	.5641	-.572524
	49	43.57	.4357	-.830801
	94	32.12	.3212	-1.13569
	166	27.89	.2789	-1.27690
AVERAGE	0	100	1	0
	22	61.89667	.6189667	-.486202
	49	48.01333	.4801333	-.736268
	94	37.11333	.3711333	-.997182
	166	30.85333	.3085333	-1.17836

TABLE 4. ALPHA-CAROTENE LOSS
AUTOXIDATION - 20 C
DEHYDRATED CARROTS

	TIME, hrs	PERCENT LOSS		
		C	C/Co	ln C/Co
REP. 1	0	100	1	0
	45	100	1	0
	81	100	1	0
	123	86.61	.8661	-.143755
	221	81.1	.811	-.209487
	303	67.32	.6732	-.395713
	373	57.39	.5739	-.555300
REP. 2	0	100	1	0
	45	100	1	0
	81	100	1	0
	123	87.66	.8766	-.131704
	221	81.44	.8144	-.205304
	303	68.31	.6831	-.381114
	373	58.59	.5859	-.534606
REP. 3	0	100	1	0
	45	100	1	0
	81	100	1	0
	123	87.5	.875	-.133531
	221	83.76	.8376	-.177215
	303	70.17	.7017	-.354249
	373	67.72	.6772	-.389789
AVERAGE	0	100	1	0
	45	100	1	0
	81	100	1	0
	123	87.25667	.8725667	-.136330
	221	82.1	.821	-.197335
	303	68.6	.686	-.377025
	373	61.23333	.6123333	-.493232

TABLE 5. ALPHA-CAROTENE LOSS
AUTOXIDATION - 40 C
DEHYDRATED CARROTS

	TIME, hrs	PERCENT LOSS		
		C	C/Co	ln C/Co
REP. 1	0	100	1	0
	7	101.99	1.0199	.0197046
	23	93.61	.9361	-.066033
	32	86.62	.8662	-.143639
	56	75.19	.7519	-.285152
	85	68.18	.6818	-.383019
	153	54.09	.5409	-.614521
REP. 2	0	100	1	0
	7	98.29	.9829	-.017248
	23	86.26	.8626	-.147804
	32	81.19	.8119	-.208378
	56	72.99	.7299	-.314848
	85	67.74	.6774	-.389493
	153	48.43	.4843	-.725051
REP. 3	0	100	1	0
	7	96.61	.9661	-.034488
	23	82.87	.8287	-.187897
	32	80.79	.8079	-.213317
	56	67.65	.6765	-.390823
	85	66.86	.6686	-.402569
	153	47.6	.476	-.742337
AVERAGE	0	100	1	0
	7	98.96333	.9896333	-.010677
	23	87.58	.8758	-.133911
	32	82.86667	.8286667	-.188445
	56	71.94333	.7194333	-.330274
	85	67.59333	.6759333	-.391694
	153	50.04	.5004	-.693970

TABLE 6. ALPHA-CAROTENE LOSS
AUTOXIDATION - 60 C
DEHYDRATED CARROTS

	TIME, hrs	PERCENT LOSS		
		C	C/Co	ln C/Co
REP. 1	0	100	1	0
	22	75.52	.7552	-.280773
	49	44.87	.4487	-.801401
	94	34.53	.3453	-1.06334
	166	27.81	.2781	-1.27977
REP. 2	0	100	1	0
	22	66.63	.6663	-.406015
	49	36.05	.3605	-1.02026
	94	31.93	.3193	-1.14162
	166	27.23	.2723	-1.30085
REP. 3	0	100	1	0
	22	63.75	.6375	-.450201
	49	31.54	.3154	-1.15391
	94	30.36	.3036	-1.19204
	166	27.03	.2703	-1.30822
AVERAGE	0	100	1	0
	22	68.63333	.6863333	-.378996
	49	37.48667	.3748667	-.991859
	94	32.27333	.3227333	-1.13234
	166	27.35667	.2735667	-1.29628

TABLE 7. LINEAR REGRESSION ANALYSIS
ALPHA- AND BETA-CAROTENE AUTOXIDATION
DEHYDRATED CARROTS

ALPHA-CAROTENE				
	20 C	40 C	60 C	
REP. 1				
slope	-.001759	-.004264	-.01646	
interc.	-.01605	.006484	-.0289	
correl.	.9645	.9774	.9874	
REP. 2				
slope	-.001704	-.004801	-.02089	
interc.	-.0133	-.01718	.01906	
correl.	.9705	.9564	.9966	
REP. 3				
slope	-.001288	-.00468	-.02364	
interc.	-.03161	-.04362	.02485	
correl.	.9409	.9632	.9955	
RATE CONSTANT				
average	.001584	.004582	.02033	
BETA-CAROTENE				
	20 C	40 C	60 C	
REP. 1				
slope	-.001877	-.00534	-.01334	
interc.	.02851	-.03264	-.01192	
correl.	.9575	.9483	.9967	
REP. 2				
slope	-.001982	-.005563	-.01441	
interc.	-.00094	-.05633	-.086	
correl.	.9791	.9463	.8722	
REP. 3				
slope	-.002222	-.006068	-.01668	
interc.	.02123	-.09608	-.07304	
correl.	.9668	.9306	.9269	
RATE CONSTANT				
average	.002027	.005657	.01481	

TABLE 8. GC RESPONSE FOR ALL VOLATILE PRODUCTS
FROM BONDS 1 TO 4
DEHYDRATED CARROTS - 20 C

	TIME, HRS	BOND 1	BOND 2	BOND 3	BOND 4
REPLICATE	0	0	0	0	0
No. 1	45	74986	12780	24380	162740
	81	158038	56049	121984	316511
	123	169687	93229	131913	461313
REPLICATE	0	0	0	0	0
No. 2	45	36302	12780	24380	162740
	81	114230	56049	48244	316511
	123	140803	93229	58173	461313
REPLICATE	0	0	0	0	0
No. 3	45	57573	12780	24380	162740
	81	138063	56049	85114	316511
	123	179285	93229	94900	461313
AVERAGE	0	0	0	0	0
	45	56287	12780	24380	162740
	81	136777	56049	85114	316511
	123	163258.3	93229	94995.33	461313

TABLE 9. GC RESPONSE FOR ALL VOLATILE PRODUCTS
FROM BONDS 1 TO 4
DEHYDRATED CARROTS - 40 C

	TIME, HRS	BOND 1	BOND 2	BOND 3	BOND 4
REPLICATE	0	0	0	0	0
No. 1	7	33354	27595	66179	195911
	23	55837	56643	89360	617663
	32	72405	196698	112658	1170114
	56	83646	359218	455168	1962335
	85	129532	540478	526626	2135315
	120	142308	654073	599044	2283724
REPLICATE	0	0	0	0	0
No. 2	7	12989	17556	42727	158537
	23	31944	45988	74546	471593
	32	42160	105357	95852	793346
	56	48453	187933	114911	1527666
	85	97758	422979	157805	1722092
	120	194436	538183	209419	1912152
REPLICATE	0	0	0	0	0
No. 3	7	21007	22576	54453	121162
	23	36433	38348	86272	325522
	32	43473	113017	108574	762624
	56	49081	219840	370222	1525895
	85	96677	351793	551392	1709598
	120	136823	547742	615670	1727504
AVERAGE	0	0	0	0	0
	7	22450	22575.67	54453	158536.7
	23	41404.67	46993	83392.67	471592.7
	32	52679.33	138357.3	105694.7	908694.7
	56	60393.33	255663.7	313433.7	1671965.
	85	107989	438416.7	411941	1855668.
	120	157855.7	579999.3	474711	1974460

TABLE 10.GC RESPONSE FOR ALL VOLATILE PRODUCTS
FROM BONDS 1 TO 4
DEHYDRATED CARROTS - 60 C

	TIME, HRS	BOND 1	BOND 2	BOND 3	BOND 4
REPLICATE	0	0	0	0	0
No. 1	22	25964	193768	180275	499228
	49	47200	507896	635760	1384254
	94	49653	571981	983540	1778882
	166	58568	618931	1220020	1947494
REPLICATE	0	0	0	0	0
No. 2	22	19935	264781	157960	614973
	49	26212	394804	430535	1020096
	94	30728	490298	809415	1467566
	166	31255	506662	972675	1617159
REPLICATE	0	0	0	0	0
No. 3	22	21588	335793	149476	730718
	49	29013	483983	529656	1879243
	94	40061	717551	892986	2179001
	166	40672	752798	1092856	2300808
AVERAGE	0	0	0	0	0
	22	22495.67	264780.7	162570.3	614973
	49	34141.67	462227.7	531983.7	1427864.
	94	40147.33	593276.7	895313.7	1808483
	166	43498.33	626130.3	1095184.	1955154.

TABLE 11. LINEAR REGRESSION FOR Bi/B3

DEHYDRATED CARROTS - 20 C				
		B1/B3	B2/B3	B4/B3
REP. 1	slope	.8686	.625	2.2915
	interc.	53662	-3957	100966
	correl.	.9991	.851	.8318
REP. 2	slope	3.1242	2.2764	8.3959
	interc.	-39100	-45229	-52533
	correl.	.9982	.9643	.9539
REP. 3	slope	1.5844	.9894	3.6325
	interc.	17029	-13393	66034
	correl.	.9561	.8815	.8639

DEHYDRATED CARROTS - 40 C				
		B1/B3	B2/B3	B4/B3
REP. 1	slope	.1418	.9236	3.5386
	interc.	39500	5218	331627
	correl.	.7686	.9086	.8804
REP. 2	slope	.7099	3.5946	11.6316
	interc.	-22318	-193316	-469574
	correl.	.9446	.9093	.5997
REP. 3	slope	.1202	.6218	3.0894
	interc.	21197	3501	165473
	correl.	.8413	.9662	.9014

DEHYDRATED CARROTS - 60 C				
		B1/B3	B2/B3	B4/B3
REP. 1	slope	.04767	.7051	1.9867
	interc.	17370	66649	141083
	correl.	1	1	1
REP. 2	slope	.02303	.477	1.4863
	interc.	16297	189431	380200
	correl.	1	1	1
REP. 3	slope	.01953	.3898	3.021
	interc.	18669	277529	279151
	correl.	1	1	1

TABLE 12. RATE CONSTANT FOR THE
FORMATION OF VOLATILES
FROM CLEAVAGE AT BONDS 1, 2, 3 & 4
OF BETA-CAROTENE IN DEHYDRATED CARROTS.

	20 C	40 C	60 C
BOND 1	.0003679	.0001431	.0001888
	.000428	.0002371	.0001142
	.0004457	.0001407	.0000653
average	.0004139	.0001736	.0001228
BOND 2	.0002648	.0009324	.0027925
	.0003119	.0012006	.002366
	.0002783	.0007281	.0013031
average	.000285	.0009537	.0021539
BOND 3	.0004236	.0010095	.0039605
	.000137	.000334	.0049593
	.0002813	.0011709	.003343
average	.0002806	.0008381	.0040876
BOND 4	.0009707	.0035722	.007868
	.00115	.003885	.007371
	.0010218	.0036174	.0100992
average	.0010475	.0036915	.0084461

TABLE 13. ACTIVATION ENERGY
ALPHA- AND BETA-CAROTENE LOSS
DEHYDRATED CARROTS

	ALPHA-CAROTENE	BETA-CAROTENE
REP. 1		
slope	-5423	-4783
interc.	12.07	10.05
correl	.9757	1
REP. 2		
slope	-6082	-4836
interc.	14.29	10.27
correl	.9814	.9998
REP. 3		
slope	-7071	-4910
interc.	17.4	10.63
correl	.9896	.9985
ACTIVATION ENERGY (cal/mol)		
	12221.05	9558.553
FREQUENCY FACTOR		
	2162265.	30232.32

TABLE 14. ACTIVATION ENERGY
FORMATION OF VOLATILES FROM
BONDS 1, 2, 3 AND 4
DEHYDRATED CARROTS

	BOND 1	BOND 2	BOND 3	BOND 4
REP. 1				
slope	1688	-5746	-5419	-5123
interc.	-13.85	11.38	10.63	10.61
correl	.5089	1	.9731	.9892
REP. 2				
slope	3211	-4970	-8649	-4555
interc.	-18.68	8.97	20.31	8.85
correl	.9905	.9771	.9014	.9803
REP. 3				
slope	4698	-3780	-6048	-5592
interc.	-23.79	4.76	12.5	12.21
correl	.9938	.9891	.9974	.9995

ACTIVATION ENERGY
(cal/mol)

-6313.82 9536.842 13234.21 10046.05

FREQUENCY FACTOR

7.028e-9 4315.636 1943498. 38432.80

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