EFFECTS OF SYNTHETIC FOOD COLORANTS ON SINGLET OXYGEN OXIDATION OF FOODS

DISSERTATION

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By

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To My Parents

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FIELDS OF STUDY

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TABLE OF CONTENTS

DEDICATION ii

ACKNOWLEDGMENTS iii

VITA iv

I. INTRODUCTION 1

II. Literature Review 4

1. Role and Use of Food Colorants 4

2. Types of Colorants Used in Foods 5

2.1. Natural Colorants 5

2.2. Synthetic Colorants 7

3. Colorant Photosensitized Oxidation of Food 14

4. Mechanisms of Photosensitized Lipid Oxidation 14

5. Chemistry of Triplet and Singlet Oxygens 19

5.1. Molecular Orbital of Triplet Oxygen and Singlet Oxygen 19

5.2. Singlet Oxygen Formation 25

5.2.1. Photosensitization 25

5.2.2. Electrical Discharge 25

5.2.3. Chemical Reaction 25

5.2.4. Enzymatic Reaction 25

5.3. Reaction Mechanisms of Singlet Oxygen with Lipid ... 26

5.4. Detection of Singlet Oxygen 26
5.4.1. Chemical Trap 27
5.4.2. Luminescence 27
5.4.3. ESR Spectroscope 28
5.4.4. Headsapce Oxygen Depletion 28
6. Quenching Mechanisms and Quenchers of Singlet Oxygen 29
7. Steady-State Kinetics of Singlet Oxygen Oxidation 30
8. Roles of Ascorbic Acid in Human Health 38
8.1. Collagen 38
8.2. Metabolism of Tyrosine, Triptophan, and Folic Acid .. 38
8.3. Iron Utilization 39
8.4. Metabolism of Fats and Oils 39
8.5. Antioxidant in the Protection of Vitamin A and E 39
8.6. Strength of Capillary Walls and Blood Vessels 39
8.7. Removal of Ammonia in the Deamination of Protein and Peptides 40
9. Applications of Ascorbic Acid in Foods 40
9.1. Use as a Nutrient 40
9.2. Use as a Processing Aid 41
10. Factors Affecting Oxidation of Ascorbic Acid 42
10.1. pH 44
10.2. Light 44
10.3. Metal Ions 45
10.4. Oxygen 45

10.5. Temperature 46
11. Ascorbic Acid Analysis of Foods 46
12. Kinetics of Ascorbic Acid Degradation 48

III. METERIALS AND METHODS 51
1. Materials 51
2. Sample Preparation for the Reproducibility of Gas Chromatographic Headspace Oxygen Analysis 51
3. Effects of Synthetic Colorants on the Oxidation of Soybean Oil during Storage 52
3.1. Sample Preparation 52
3.2. Storage of Sample Bottles 52
3.2.1. Light Box for Sample Storage 52
3.2.2. Sample Storage Conditions 54
3.3. Headspace Oxygen Analysis 54
4. Sample Preparation and Analyses for the Quenching Mechanisms and Kinetics of α-Tocopherol on the Red No. 3 Photosensitized Oxidation of Soybean Oil 56
5. Sample Preparation and Analyses for the Quenching Mechanisms and Kinetics of β-Carotene on Red No. 3 Photosensitized Oxidation of Soybean Oil 56
6. Effects of Red No. 3 on the Oxidation of Ascorbic Acid 57
6.1. Preparation of Buffer Solution 57
6.2. Sample Preparation for the Spectrophotometric Determination of Ascorbic Acid 58

6.3. Sample Preparation for the Effect of pH on the Color Stability of Red No. 3 58
6.4. Sample Preparation for the Effects of Red No. 3 on the Oxidation of Ascorbic Acid 59
7. Statistical Analysis 59

IV. RESULTS AND DISCUSSION 60
1. Reproducibility of Headspace Oxygen Analysis by Gas Chromatography 60
2. Model Sample for Studying Lipid Oxidation in the Presence of Water Soluble Synthetic Colorants 62
3. Effect of Oil Concentrations on the Headspace Oxygen Depletion of Red No. 3 Photosensitized Oxidation of Soybean Oil 64
4. Effects of FD&C Colorants on the Oxidation of Soybean Oil under Light 66
5. Quenching Mechanisms and Kinetics of α-Tocopherol on the Red No. 3 Photosensitized Oxidation of Soybean Oil ... 70
6. Quenching Mechanisms and Kinetics of β-Carotene on the Red No. 3 Photosensitized Oxidation of Soybean Oil ... 76
7. Determination of Ascorbic Acid Contents by Spectrophotometry 83
8. Effects of pH on the Oxidation of Red No. 3 and Ascorbic Acid in the Presence of Red No. 3 under Dark and Light 83

9. Determination of the Reaction Rate Constants of Singlet Oxygen Oxidation of Ascorbic Acid 88
10. Determination of the Reaction Rate Constants of Singlet Oxygen Oxidation of Ascorbic Acid by Headspace Oxygen Analysis 90

V. CONCLUSIONS 97

LIST OF REFERENCES 100

LIST OF TABLES

TABLES PAGE

1. Natural food colorants used in the United States 6

2. Certified synthetic colorants and usage levels in foods 10

3. Per capita daily intake of straight FD&C colorants in milligrams 11

4. Per capita daily intake of lakes of FD&C colorants in milligrams 12

5. Lifetime of singlet oxygen in solvents 23

6. Coefficient of variation for the gas chromatographic headspace oxygen analysis of sample bottles 61

7. Effect of 100 ppm Red No. 3, Eosin B, rose bengal, and methylene blue on the headspace oxygen depletion of the oil sample bottles under light for 2 hr 63

8. Effect of soybean oil concentrations on the headspace oxygen depletion of sample bottles containing 20 ppm Red No. 3 in acetone under light for 1 hr 66

9. Effects of FD&C colorants on the headspace oxygen of soybean oil under light storage 68

10. Effects of soybean oil and α-tocopherol concentrations on the headspace oxygen depletion of samples containing 20 ppm Red No. 3 70

11. The intercepts and slopes of the plots of $[AO_2]^{-1}$ vs. $[A]^{-1}$ of Fig. 16 to determine the quenching mechanism and rate of α-tocopherol on the Red No. 3 photosensitized oxidation of soybean oil.....73

12. Effects of soybean oil and β-carotene concentrations on the headspace oxygen depletion of sample bottles containing Red No. 3 and soybean oil under light for 1 hr.....77

13. The intercepts and slopes of the plots of $[AO_2]^{-1}$ vs. $[A]^{-1}$ of Fig. 18 to determine the quenching mechanism and rate of β-carotene on the Red No. 3 photosensitized oxidation of soybean oil.....78

14. The effects of β-carotene on the formation of singlet oxygen in the presence of 20 ppm Red No. 3 under light for 1 hr.....80

15. The effect of pH on the 40 ppm Red No. 3 solution under dark and light for 1 hr.....85

16. The effects of 40 ppm Red No. 3 on the ascorbic acid oxidation at pH 4.0, 5.6 and 7 under light for 1 hr.....87

17. The effect of pH on the 50 ppm ascorbic acid solution in the presence of 40 ppm Red No. 3 under dark for 1 hr.....88

18. The intercepts and slopes of the plots at pH 4.0, 5.6, and 7.0 of Figs. 21, 22, and 23 to determine the reaction rate constants of singlet oxygen oxidation of ascorbic acid.....89

19. The effect of 40 ppm Red No. 3 on the headspace oxygen depletion of ascorbic acid samples under light for 1 hr.....90

LIST OF FIGURES

FIGURES PAGE

1. Certified synthetic colorants used in the U. S.....8

2. Chromophoric systems of synthetic food colorants...13

3. Excitation and deactivation of a sensitizer.....15

4. Type I and Type II pathways for excited triplet sensitizer.....17

5. Molecular orbital of triplet oxygen.....20

6. Molecular orbital of singlet oxygen.....21

7. Formation and quenching of singlet oxygen 31

8. The formation rate of oxidized compound.....33

9. Determination of singlet oxygen quenching mechanism.....34

10. The slope/intercept of the plot of Fig. 9 vs. quencher concentrations to determine quenching rate of singlet oxygen..... 35

11. Determination of triplet sensitizer quenching mechanism.....36

12. The relationship between slope/intercept of the plot of Fig. 11 vs. quencher concentrations in triplet sensitizer quenching.....37

13. Degradation mechanisms of ascorbic acid.....43

14. Diagram of light box for storage of samples.....53

15. Gas chromatograms of the headsapce oxygen content of sample bottle containing 0.01 M soybean oil and 20 ppm Red No. 3 in acetone under light for 1 hr.....65

16. Effect of α-tocopherol on the headspace oxygen depletion of soybean oil containing 20 ppm Red No. 3 under light for 1 hr at 25°C.....74

17. Regression line of slope/intercept of Fig. 16 vs. α-tocopherol concentrations.....75

18. Effect of β-carotene on the headspace oxygen depletion of soybean oil containing 20 ppm Red No. 3 under light for 1 hr at 25°C.....81

19. Regression line of slope/intercept of Fig. 18 vs. β-carotene concentrations.....82

20. Correlation of absorbances at 265 nm and ascorbic acid concentrations.....84

21. Rate of ascorbic acid loss vs. ascorbic acid concentrations at pH 4.0 in the presence of 40 ppm Red No. 3 under light for 1 hr.....93

22. Rate of ascorbic acid loss vs. ascorbic acid concentrations at pH 5.6 in the presence of 40 ppm Red No. 3 under light for 1 hr..... 94

23. Rate of ascorbic acid loss vs. ascorbic acid concentrations at pH 7.0 in the presence of 40 ppm Red No. 3 under light for 1 hr..... 95

24. Reciprocal of headspace oxygen depletion vs. ascorbic acid concentrations in the presence of 40 ppm Red No. 3 under light for 1 hr..... 96

I. INTRODUCTION

Lipid oxidation of food causes off-flavor, loss of nutrients, and production of undesirable compounds (Blume, 1987; Addis, 1990) which not only deteriorate food quality but render food products less acceptable or unacceptable to consumers (Mistry and Min, 1992). Lipid oxidation is due to free radical chain reaction which could be initiated by singlet oxygen. Singlet oxygen can react with linoleic acid approximately 1500 times faster than triplet oxygen (Rawls and Van Santen, 1970). Singlet oxygen can be produced by photosensitizers in the presence of light and triplet oxygen (Foote, 1976). Natural colors and pigments such as riboflavin, chlorophyll, and myoglobin derivatives have been reported as photosensitizers (Bradley and Min, 1992). Synthetic food colorants, which have been used to improve the appearance of foods, may act as photosensitizers due to their highly conjugated molecular structures. Therefore, the wide use of these colorants may influence the quality and safety of foods.

Ascorbic acid is an important compound in human health. Its biochemical functions include (1) the formation and maintenance of collagen, (2) the metabolism of amino acid tyrosine, tryptophan and folic acid, (3) the absorption and movement of iron, (4) the

metabolism of fats and lipids, and cholesterol control, (5) the protection of vitamins A and E as an antioxidant, (6) the strength of capillary walls and blood vessels, and (7) the removal of ammonia in the deamination of proteins and peptides (Ensminger et al., 1994). Foods such as vegetables, citrus fruits, green tea, green soybean, and papaya are rich sources of ascorbic acid. Ascorbic acid is frequently used as an antioxidant in the processed foods such as ham, bacon, jam, and juice to increase the oxidative stability of products. Of all the vitamins, ascorbic acid is the most unstable (Ensminger et al., 1994). It is easily destroyed during harvesting, processing, cooking, and storage (Ensminger et al., 1994). Therefore, the content of ascorbic acid in food products is frequently used as an index of food quality (Tsumura et al., 1993). The degradation of ascorbic acid is affected by temperature, pH, oxygen, light and metal ions (Sattar et al., 1977; Eison-Perchonok and Downes, 1982; Sahbaz and Somer, 1993). There have been many studies on the effects of temperature, pH, oxygen, and metal ions and light on the degradation of ascorbic acid in foods. The information about the photosensitized oxidation of ascorbic acid in the presence of FD&C colorants is not available.

Preventing singlet oxygen oxidation is important to maintain the quality of foods during processing and storage. α-Tocopherol and β-carotene are natural compounds which have antioxidant activities on free radical and/or singlet oxygen oxidation of foods. β-Carotene is an effective singlet oxygen quencher at diffusion-

controlled rate (Foote, 1976). The effects of synthetic food colorants on the singlet oxidation of lipid, and the quenching effects, mechanisms and kinetics of α-tocopherol and β-carotene on the synthetic food colorants sensitized singlet oxygen oxidation of lipid have not been studied.

The objectives of research are (1) to develop a stable solution of the hydrophilic colorants and hydrophobic oil, (2) to design a light box to provide an uniform light intensity to the samples during storage, (3) to study the effects of synthetic food colorants on the oxidation of soybean oil, (4) to study the quenching effects, mechanisms and kinetics of α-tocopherol and β-carotene on the synthetic food colorant photosensitized singlet oxygen oxidation of soybean oil, and (5) to study the effects of pH and Red No.3 on the oxidation of ascorbic acid during storage.

II. LITERATURE REVIEW

1. Role and Use of Food Colorants

Color is an important component in the flavor quality of foods and can affect consumer's acceptance of foods. The use of colorants to camouflage the defects of foods is not allowed by FDA's regulations. The functions of colorants in foods are as follows (Walford, 1980):

(1) To give an attractive appearance to those products of which the natural colors would be destroyed during processing or storage conditions.

(2) To impart color to those processed foods naturally with little or no colors such as soft drinks, confectionery and ice-creams.

(3) To fortify the intensity of natural colors where perceived to be weak.

(4) To normalize batch-to-batch color inconsistency of raw materials which may come from different sources.

2. Types of Colorants Used in Foods

Food colorants are substances which can color a food product when they are used in the food. They are regulated as food additives by FDA. Food colorants are customarily divided into natural and synthetic.

2.1. Natural Colorants

Natural colors are those compounds obtained from natural sources, including vegetables, animals, or minerals. Natural colorants are exempt from certification. The "natural-identical" colors are the synthetic counterparts of those naturally occurring pigments. Natural-identical colors are all exempt from certification, but the maximum usage levels of some colorants such as canthaxanthin and β-apo-8'-carotenal are regulated by FDA (Meggos, 1984). The major natural food colorants currently used In the United States are listed in Table 1.

Table 1 - Natural food colorants used in the United States

Colorant	Hue	Major chromophore	Sources
Annatto	orange-yellow	bixin seed of a tree	*Bixa orellano*
Turmeric	yellow-golden brown	curcumin	a plant, *Curcuma long L.*
Beet juice	deep red, red-purple	betanin	beet, *Beta vulgaris ruba*
Paprika	red - orange	capsanthin & capsorubin	red pepper, *Capsicum annum L.*
Cochineal /Carmine	bright red-purple red	carminic acid	insect, *Dactylopius coccus costa* or *Coccus cati*
Grapeskin extract	wine-red to red purple	anthocyanins	grapes, *Vitis labrusca* or *Vitis vinifera*
Caramel	dark brown	complex mixture	heating of carbohydrate

The safety concern and exemption of tedious procedures for certification are the major reasons why natural colors are used. Nevertheless, the applications of natural colors in foods have some of the following defects and disadvantages (Walford, 1980).

(1) Fastness deficiencies
(2) Restricted shade range
(3) High cost in use
(4) Low effective agent concentration
(5) Considerable batch to batch variation
(6) Distinctive aroma or spicy taste

(7) Sensitive to the environmental changes such as pH and temperature

(8) Sanitation concern and liable to microbial spoilage

(9) Unstable supplying sources

2.2. Synthetic Colorants

Synthetic colorants have been developed to compensate or minimize the defects experienced in the use of natural colorants. Synthetic colorants are superior to natural colorants in

(1) Tinctorial power

(2) Consistency of the strength, range and brilliance of shade

(3) Stability

(4) Easy application

(5) Cost effectiveness

There are nine synthetic food colorants approved by FDA for use in foods in the United States (Fig. 1). The applications of synthetic colorants in major food products are listed in Table 2. The amounts of daily intake of synthetic colorants per capita in the United States are listed in Tables 3 and 4.

The synthetic colorants are classified into five chromophoric groups: (1) Monoazo, Diazo, Triazo, (2) Triarylmethane, (3) Xanthene, (4) Quinoline, and (5) Indigoid and are shown in Fig. 2 (Coulson, 1980).

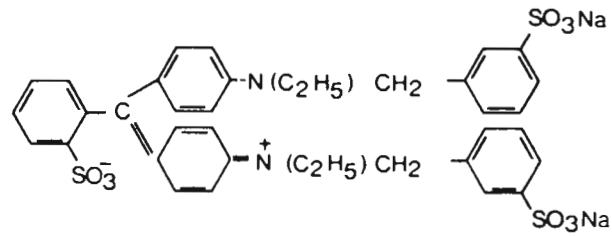
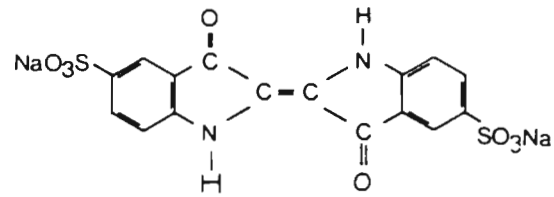
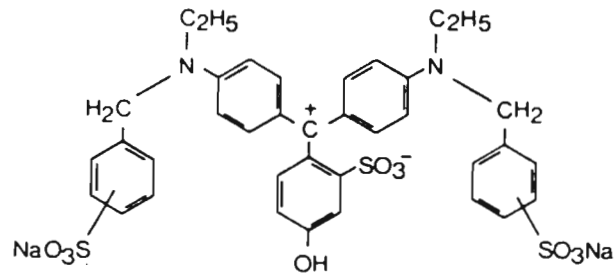
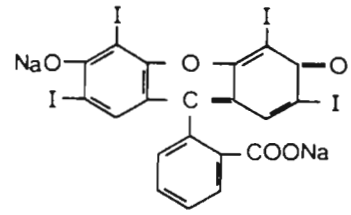
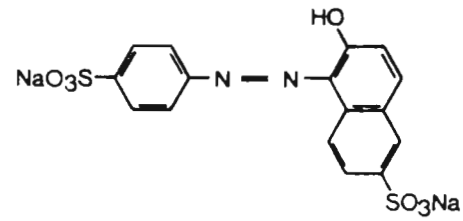
Name	Structure
FD&C Blue No. 1 Brilliant Blue FCF CI 42090 Listed in 1929	
FD&C Blue No. 2 Indigo Carmine (Indigotine) CI 73015 Listed in 1907	
FD&C Green No. 3 Fast Green FCF CI 42090 Listed in 1927	
FD&C Red No. 3 Erythrosine CI 45430 Listed in 1907	

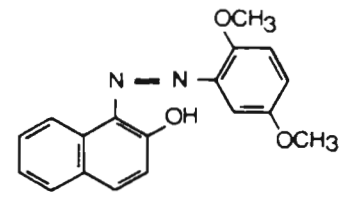
Fig. 1 - Certified synthetic colorants used in the U. S.

Fig. 1- Continued

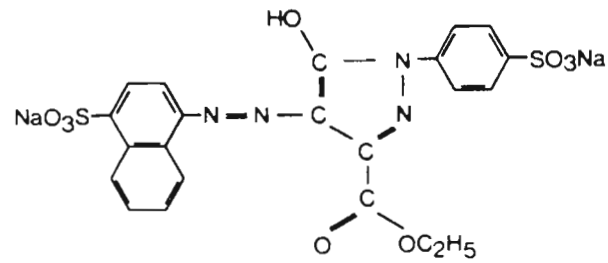
FD&C Yellow No. 6
Sunset Yellow FCF
CI 15985
Listed in 1929



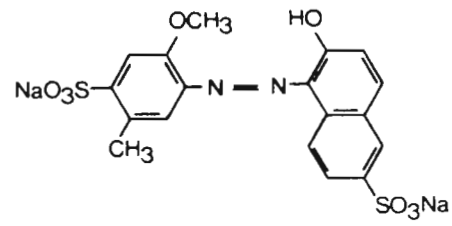
Citrus Red No. 2
CI 12156
Listed in 1959



Orange B
CI 19235
Listed in 1966



FD&C Red No. 40
Allura Red®
CI 16035
Listed in 1971



FD&C Yellow No. 5
Tartrazine
CI 19140
Listed in 1916

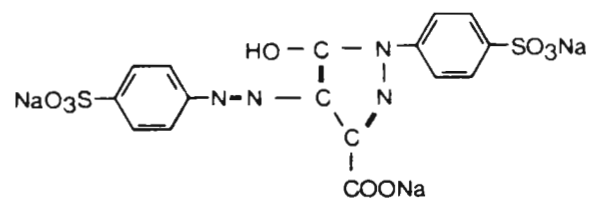


Table 2 - Certified synthetic colorants and usage levels in foods

Category	Level of colorants used	
	Range (ppm)	Average (ppm)
Candy and confections	10-400	100
Beverages (Liquid and powdered)	5-200	75
Dessert powders	5-600	140
Cereals	200-500	350
Maraschino cherries	100-400	200
Pet foods	100-400	200
Bakery goods	10-500	50
Ice cream and sherbets	10-200	30
Sausage (surface)	40-250	125
Snack foods	25-500	200
Miscellaneous (nuts, salad dressing, gravy, spices, jams, jellies, food packages, etc.)	5-400	-

(Certified Color Industry Committee, 1968)

Table 3 - Per capita daily intake of straight FD&C colorants in milligrams

Colorants	Age		
	6-23 month	6-12 years	18-44 years
Blue No. 1	2.90	4.5	3.6
Blue No. 2	0.59	1.6	1.0
Green No. 3	0.68	1.1	0.8
Red No. 3	5.90	9.9	7.0
Red No. 40	19.00	31.0	26.0
Yellow No. 5	9.40	15.0	13.0
Yellow No. 6	6.80	14.0	11.0
Total	45.30	77.1	62.4

(USDA, 1972)

Table 4 - Per capita daily intake of lakes of FD&C colorants in milligrams

Colorants	Age		
	6-23 month	6-12 years	18-44 years
Blue No. 1 (Aluminum lake)	0.52	1.00	0.76
Blue No. 2 (Aluminum lake)	0.35	0.54	0.49
Green No. 3 (Aluminum lake)	none	none	none
Red No. 3 (Aluminum lake)	1.30	2.80	2.10
Red No. 40 (Aluminum lake)	2.20	4.90	3.80
Red No. 40 (calcium lake)	none	1.80	2.50
Yellow No. 5 (Aluminum lake)	2.20	4.30	3.00
Yellow No. 5 (Aluminum lake)	0.09	0.10	0.11
Yellow No. 6 (Calcium lake)	1.10	2.70	1.70
Total	7.80	18.10	14.50

(USDA, 1972)

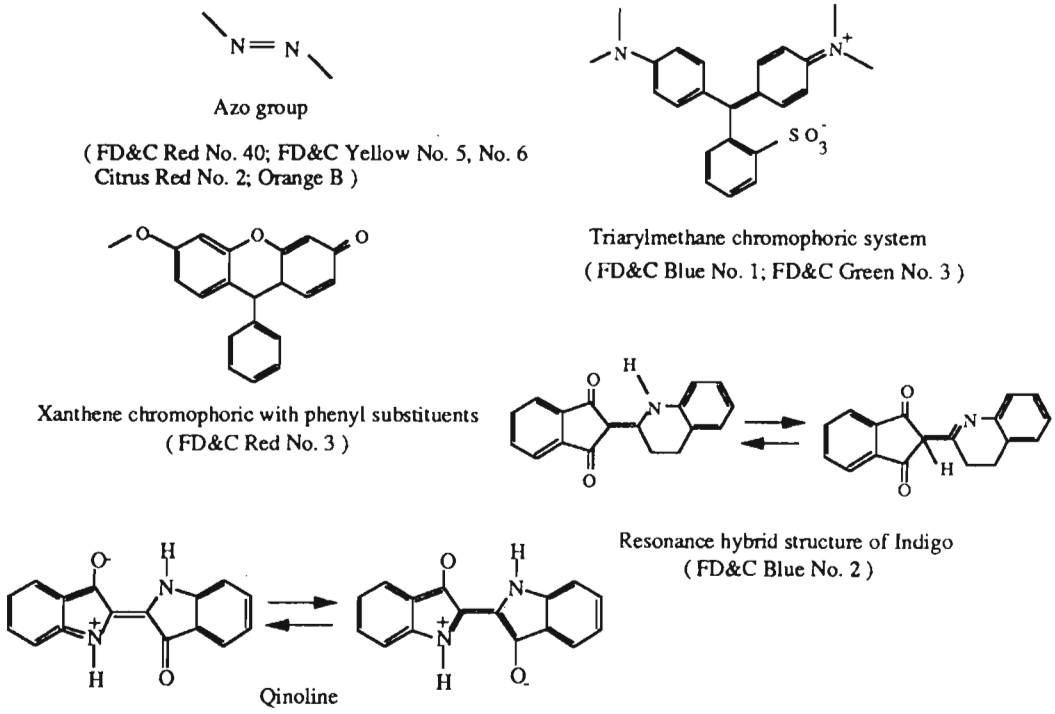


Fig. 2 - Chromophoric systems of synthetic food colorants

3. Colorant Photosensitized Oxidation of Food

Colorants can absorb visible light and express colors via their chromophoric systems (Fig. 2). Natural pigments such as chlorophylls, riboflavin, myoglobin and its derivatives were reported as photosensitizers (Rawls and Van Santen, 1970; Rogers, 1983; Whang and Peng, 1989; Jung et al., 1991; Bradly and Min, 1992). While, whether myoglobin is a sensitizer or not remains debatable (Whang and Peng, 1989). Dyes such as Eosin, methylene blue, rose bengal, and erythrosine are effective sensitizers (Chan, 1977; Suyama et al., 1983; Gloria et al., 1993).

4. Mechanisms of Photosensitized Lipid Oxidation

Lipid oxidation can be classified into (1) free radical autoxidation (2) photosensitized oxidation (3) thermal oxidation, and (4) enzymatic oxidation according to their oxidation mechanisms (Mistry and Min, 1992). Since colorants may act as photosensitizer, colorant-involved lipid oxidation is the type of photosensitized oxidation. Photosensitized oxidation is initiated by a sensitizer in the presence of light and triplet oxygen. A singlet-state sensitizer(^{1}Sen) can be excited by the visible or UV light. The excited sensitizer (^{1}Sen*) is changed to excited triplet state (^{3}Sen*) through the inter-system crossing mechanism (ISC) as shown in Fig. 3.

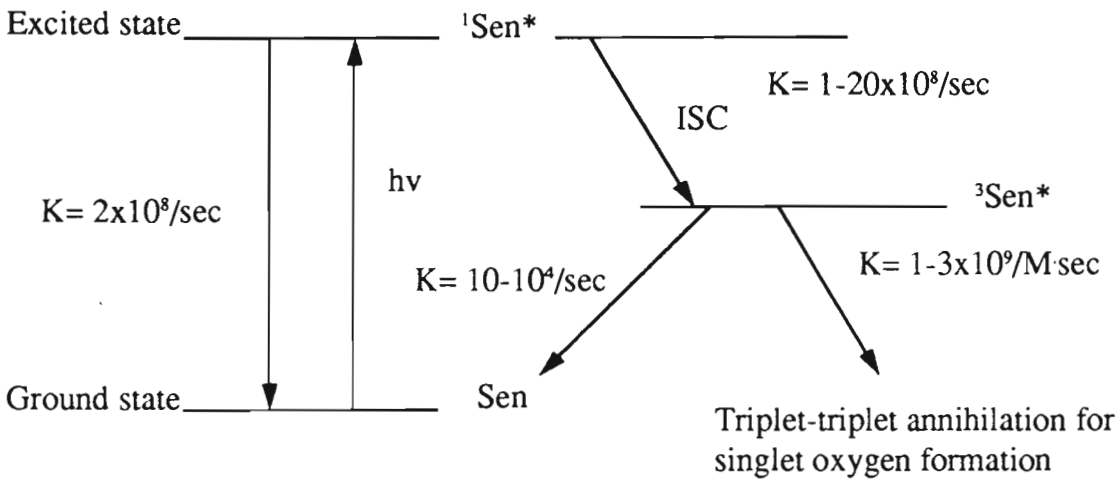


Fig. 3 - Excitation and deactivation of a sensitizer

The excited sensitizer (^{3}Sen*) can have two reaction pathways, Type I and Type II, as shown in Fig. 4. (Gollnick, 1968; Foote, 1976). ^{3}Sen* can react directly with the compound (RH) through the Type I pathway and generate radicals by mechanisms of hydrogen atom or electron transfer as follows:

$^3Sen^*(S) + RH \cdots\cdots\cdots\cdots> SH^{\cdot} + R^{\cdot}$

$^3Sen^*(S) + RH \cdots\cdots\cdots\cdots> S^{\cdot -} + RH^{\cdot +}$

The resultant radicals can initiate the formation of other reactive intermediates in the oxidative processes such as superoxide ion (O_2^- or its conjugate acid, $HO_2\cdot$), the hydroxy radical ($OH\cdot$), alkoxy radical ($RO\cdot$), and peroxy radicals ($ROO\cdot$). The formation mechanisms of these reactive intermediates are suggested as follows (Foote, 1985):

$ROOH + X \cdots\cdots\cdots\cdots> ROO\cdot + XH$

$ROOH \cdots\cdots\cdots\cdots> RO\cdot + OH^-$ (in the presence of metal ions)

$ROOH \cdots\cdots\cdots\cdots> RO\cdot + \cdot OH$

$X^- + O_2 \cdots\cdots\cdots\cdots> O_2^- + X$

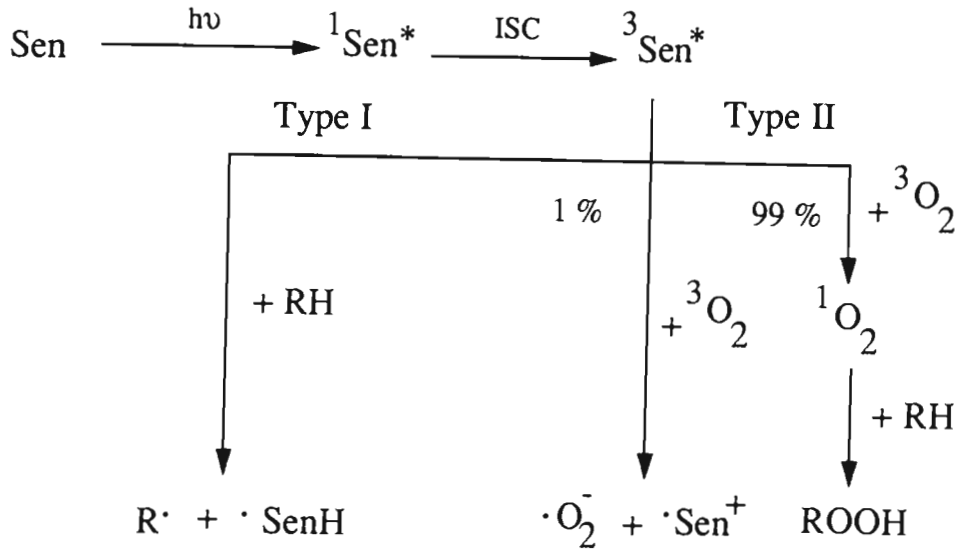


Fig. 4 - Type I and Type II pathways for excited triplet sensitizer

In Type II pathway, there is an 1% chance for the $^3Sen^*$ to react with triplet oxygen to form superoxide ion, but 99 % $^3Sen^*$ reacts with triplet oxygen to form singlet oxygen via triplet-triplet annihilation mechanism. The singlet oxygen can react with compounds such as unsaturated fatty acids of lipids to initiate lipid oxidation.

The tendency for $^3Sen^*$ to undergo Type I or Type II pathways is influenced by the reactants and reaction conditions. With respect to the reactants, the compounds which are easily reducible such as quinones or oxidizable such as phenols and amines by sensitizers favor Type I reaction. In contrast, the compounds such as olefins, dienes, and aromatic compounds, are not easily reducible or oxidizable by sensitizers favor Type II reaction. Exceptions to the generalities were reported under certain conditions (Korycka-Dahl and Richardson, 1978). The types of sensitizers also play an important part in the determination of Type I or II pathway. Riboflavin, a water soluble sensitizer, favors Type I (Korycka-Dahl and Richardson, 1977), while oil soluble chlorophylls favor Type II (Samuel and Steckel, 1974). Triplet oxygen has greater solubility in oil than in water and favors Type II reaction (Ke and Ackman, 1973; Samuel and Steckel, 1974).

5. Chemistry of Triplet and Singlet Oxygens

5.1. Molecular Orbitals of Triplet Oxygen and Singlet Oxygen

Triplet oxygen has 12 valence electrons in the molecular orbital according to the energy levels as illustrated in Fig. 5. The molecular orbital of singlet oxygen is shown in Fig. 6. The distinction between singlet oxygen and triplet oxygen is the ways how the electrons are arranged in the π^* orbitals. The energy state of oxygen molecule under magnetic field determines the singlet or triplet state of oxygen. The spin multiplicity is used to describe the electron arrangement in the molecular orbital. It is defined as 2S +1 (S is total spin quantum number). Triplet oxygen with two electrons spinning in parallel to each other has the spin multiplicity of 3. Singlet oxygen with oppositely-spinning paired electrons has the spin multiplicity of 1. According to the Hund's rule of maximum multiplicity, the arrangement of the electrons in orbitals should give maximum total spin possible or maximum number of parallel spins. Singlet oxygen can be formed in two types, Δ and Σ, but either type of singlet oxygen conforms to the Hund's rule; therefore, they are chemically unstable. Type Δ and type Σ singlet oxygen have energy levels greater than ground-state oxygen by 22.4. and 37.5 Kcal/mol, respectively. Type Σ singlet oxygen has received less attention as it is so energetic that it rapidly decays to type Δ singlet oxygen upon being produced (Korycka-Dahl and Richardson, 1978).

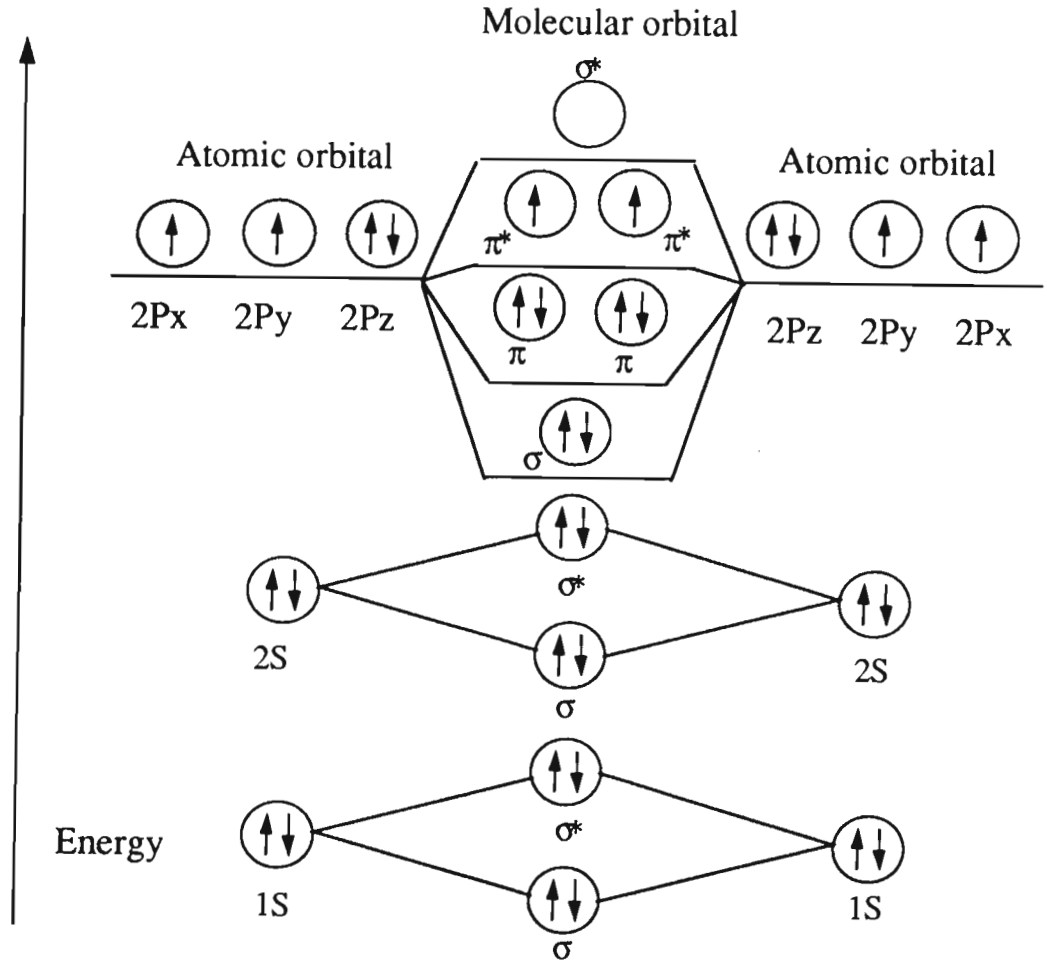


Fig. 5 - Molecular orbital of triplet oxygen

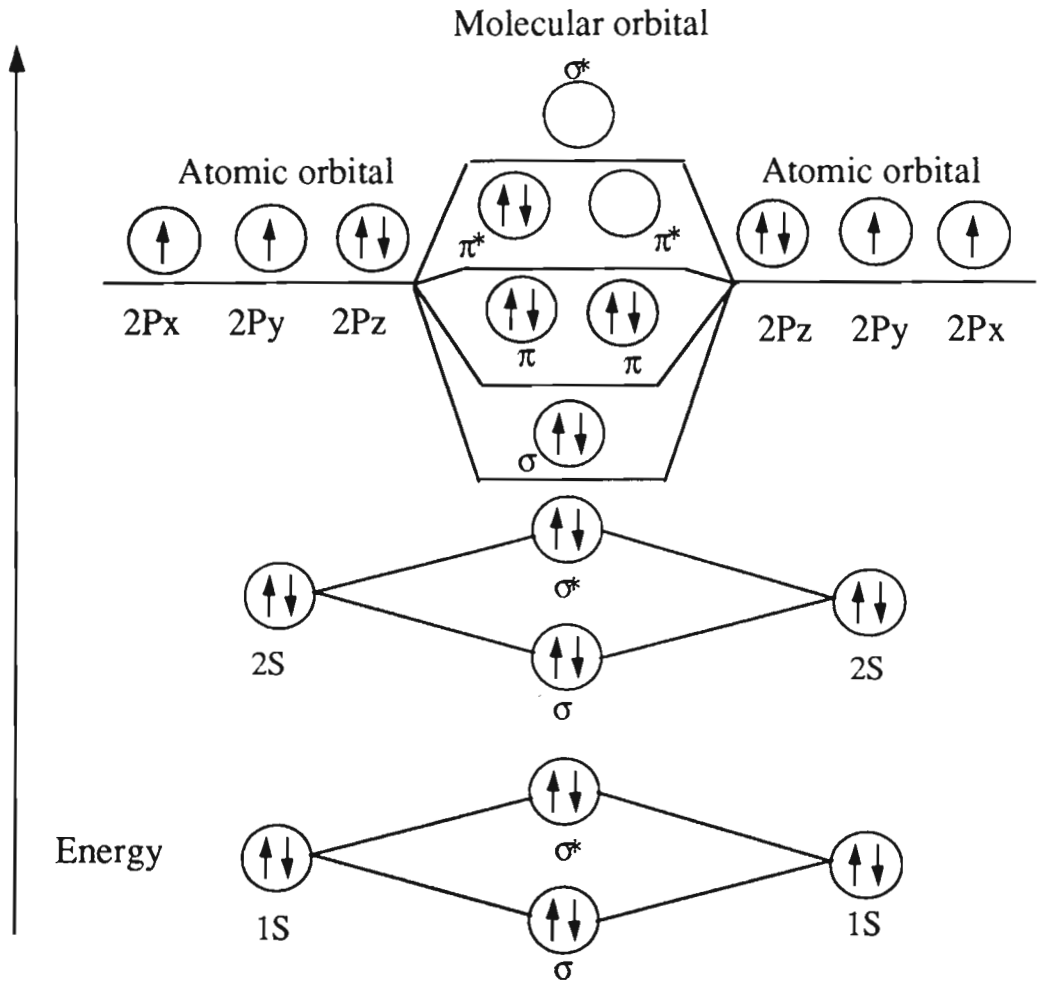


Fig. 6 - Molecular orbital of singlet oxygen

Singlet oxygen with higher energy than the triplet oxygen and a vacant orbital is energetic and electrophilic. It can easily react with electron-rich compounds such as dienes, and aromatic compounds. The reaction rate of singlet oxygen with chemical or biochemical compounds is affected by the lifetime of singlet oxygen. The lifetime of singlet oxygen is solvent dependent (Ogliby and Foote, 1983; Bradly and Min, 1992). The lifetimes of singlet oxygen in various solvents are shown in Table 5 (Gorman and Rogers, 1989). The lifetime of singlet oxygen in relatively nonpolar solvent is longer than that in water. Therefore, the singlet oxygen oxidation of lipid or lipid soluble compounds would be greater than those which are water soluble. The temperature has little effect on the lifetime of singlet oxygen and the singlet oxygen oxidation of compounds (Ogilby and Foote, 1983).

Table 5 - Lifetime of singlet oxygen in solvents

Solvent	Lifetime (μSec)
Alkanes and haloalkanes	
Pentane	22, 34.6
Hexane	30, 31.4
Cyclohexane	23, 23.0
Heptane	28, 29.5
Isooctane	37.6
Nonane	23.7
Decane	27.6
Docane	24.7
Tetradecane	24.7
Hexadecane	24.3
Dichloromethane	70, 82.9, 91, 100
Chloroform	240, 250, 265, 300
Alcohols	
Methanol	10.4
Ethanol	9.7, 10, 13, 15.3
1-Propanol	16.3
2-Propanol	22.1
1-Butanol	17.5

Table 5 - continued

1-Pentanol	17.8
1-Hexanol	17.9
1-Heptanol	18.1
1-Octanol	18.5
1-Nonanol	18.6
1-Decanol	17.8
Benzene and Halobenzenes	
Benzene	26.7, 28, 30, 31.2, 32
Chlorobenzene	42, 45, 51
Bromobenzene	43, 50
Iodobenzene	27, 35
Fluorobenzene	49
Miscellaneous Organic Media	
Acetone	39, 40, 50, 51, 65.3
Acetonitrile	54.4, 61, 68, 69
Diethyl ether	26, 30.4, 34, 35
Tetrahydrofuran	20, 30
Aqueous Media	
Water	3.1, 3.2, 4.2, 4.4, 5
Water-D_2	55, 58, 67, 100

5.2. Singlet Oxygen Formation

Singlet oxygen can be formed through the following chemical and physical mechanisms.

5.2.1. Photosensitization

Singlet oxygen can be formed by photosensitizers such as methlyene blue and chlorophyll in the presence light and triplet oxygen (Foote, 1976; Lee and Min, 1990).

5.2.2. Electrical Discharge

Singlet oxygen can be produced by exposing triplet oxygen to high-power microwave generators. Both Δ and Σ singlet oxygen are present in gaseous state (Ogryzlo, 1979).

5.2.3. Chemical Reaction

H_2O_2 reacts with hypohalite such as OCl^- and produces singlet oxygen. Hydrogen peroxide and $\cdot O_2^-$ both occurring in biological systems can react with each other to produce singlet oxygen (Foote and Wexler, 1964; Murray, 1979).

5.2.4. Enzymatic Reaction

The formation of singlet oxygen due to enzymatic reactions has been reported (Nakano et al., 1975; King et al., 1975). Many precursors such as hydrogen peroxide and $\cdot O_2^-$ for the generation of singlet oxygen were derived from enzymatic reactions (Korycka-Dahl and Richardson, 1978).

5.3. Reaction Mechanisms of Singlet Oxygen with Lipid

The reaction mechanisms for singlet oxygen to initiate oxidation of chemical or biochemical compounds are generally characterized as (1) ene reaction, (2) 1,2 cycloaddition, (3) 1,4 addition, (4) oxidation of sulfides, and (5) oxidation of phenols (Corey and Taylor, 1964; Chan, 1977). The ene reaction and oxidation of phenols are the most predominant reaction during singlet oxygen oxidation of foods (Bradley and Min, 1992).

Compounds formed by singlet oxygen oxidation of lipid have been reported (Frankel, 1985). The oxidized products of methyl oleate showed equal formation of 9- and 10-hydoperoxides but no formation of 8- and 11-hydroperoxides, which are formed in a free radical autoxidation. This is due to the direct attack of singlet oxygen on the double bonds of methyl oleate. The ene reaction can also cause steric rearrangement, from cis to trans, of the fatty acid. The volatile compounds of pure hydroperoxides of methyl oleate, linoleate, and linolenate from singlet oxygen oxidation are also different from those from triplet oxygen oxidation (Frankel, 1982; Frankel et al., 1991).

5.4. Detection of Singlet Oxygen

A variety of methods have been used for the detection of singlet oxygen and reported with respect to their applications in the determination of oxidation mechanisms in foods or biological systems.

5.4.1. Chemical Trap

Singlet oxygen can be chemically trapped by certain compounds. Furans, dienes, olefins, and aromatic compounds such as anthracene derivatives, have been used as the traps for singlet oxygen detection. Some of the oxidized compounds may be produced from other reaction pathways other than singlet oxygen oxidation (Foote, 1985). The success and usefulness of this method strictly depend on the specificity of the trap compounds for singlet oxygen and the unique formation pathway of these oxidized compounds. 5-α-Hydroperoxide cholesterol was reported as a distinctive compound formed by the singlet oxidation of cholesterol (Ansari and Smith, 1979).

5.4.2. Luminescence

Two luminescence processes of singlet oxygen molecules have been reported as the following:

$$2^1O_2 \xrightarrow{h\upsilon} \text{634 and 703 nm}$$

$$^1O_2 \xrightarrow{h\upsilon} \text{1270 nm}$$

This method provides a direct observation of the presence of singlet oxygen (Ogilby and Foote, 1983; Hurst and Schuster, 1983). However, these luminescence mechanisms are considered to be very inefficient partly due to the fact that the infrared emission

(1270 nm) is very difficult to detect especially at low concentration of singlet oxygen (Foote, 1985).

5.4.3. ESR Spectroscope

Electron spin resonance (ESR) is a highly sensitive analytical method for detecting free radical. Even though singlet oxygen is not a radical, singlet oxygen can still be detected by the technique indirectly via formation of a stable radical intermediate. The amine, 2,2,6,6-tetramethyl-4-piperidone (TMPD), has been used to react with singlet oxygen to form a stable nitroxide radical, 2,2,6,6-tetramethyl-4-piperidone-N-oxyl (TAN or TEMPONE) which can be detected by ESR (Whang and Peng, 1988; Bradly and Min, 1992). For effective analysis by ESR, the constant free radical concentration is required to maintain over 10^{-8} M. The concentration of 10^{-6} to 10^{-5} M is necessary for reasonable spectral resolution, which is a limitation of the technique.

5.4.4 Headsapce Oxygen Depletion

Singlet oxygen is produced through triplet sensitizer and triplet oxygen annihilation in the Type II pathway of photosensitzation. As singlet oxygen reacts compounds such as unsaturated fatty acids in a sample bottle, the triplet oxygen in the headspace of sample bottle will decrease. Therefore, the analysis of headspace oxygen can be used to evaluate the

formation of singlet oxygen in the presence and absence of sensitizer during storage under dark and light.

6. Quenching Mechanisms and Quenchers of Singlet Oxygen

There are two mechanisms, energy transfer and charge transfer, for singlet oxygen quenching (Foote, 1979; Gorman, 1992). Energy transfer singlet oxygen quenching is due to vibrational energy transfer to solvent or quenchers such as β-carotene. Charge transfer singlet oxygen quenching is due to chemical reaction with compounds such as amines, sulfines, azo-methane dyes, and bilirubin. The energy transfer mechanism requires that a quencher molecular has energy less than singlet oxygen. The energy transfer mechanism is more effective than the charge transfer mechanism. The maximum rate constants of energy transfer reaction and charge transfer reaction are about $2x10^{10}$ and 10^9 $M^{-1}Sec^{-1}$, respectively (Foote, 1979). Tocopherol can quench singlet oxygen by both energy transfer and charge transfer mechanisms with the rates of $4.6x10^7$ and $6.8x10^8 M^{-1}Sec^{-1}$, respectively in methylene blue photosensitization in methanol (Foote, 1976). β-Carotene was reported as very effective singlet oxygen quencher reacting at diffusion control rate of $1.3x10^{10} M^{-1}Sec^{-1}$ (Wilkinson, 1978; Foote, 1979). The quenching of singlet oxygen by β-carotene in chlorophyll photosensitized lipid oxidation has been studied. β-Carotene exhibited quenching effect on singlet oxygen but not on

chlorophyll during the light storage of soybean oil (Lee and Min, 1988). The singlet oxygen quenching effects of carotenoids such as leutein (10: numbers of double bonds), zeaxanthin (11), lycopene (11), isozeaxanthin (11), and astaxanthin (13) have been studied. The singlet oxygen quenching rate of the carotenoids increased in line with the increase of the number of the conjugated double bonds of the carotenoids (Lee and Min, 1990). β-Carotene is sensitive to light exposure. The formation of β-ionone and other decomposed products of β-carotene has been reported (Gl'oria et al., 1993). Tocopherols were reported to protect β-carotene by scavenging free radicals produced in the singlet oxygen oxidation process (Choe and Min, 1992). Metal chelates such as Nickel chelates was reported as effective singlet oxygen quenchers in chlorophyll photosensitzed oxidation of soybean oil (Lee and Min, 1991).

7. Steady-State Kinetics of Singlet Oxygen Oxidation

Steady-state kinetics has been established to study the quenching rate of quenchers with singlet oxygen or the reaction rate of substrates with singlet oxygen (Foote, 1979; Jung et al., 1991). The formation and quenching mechanisms of singlet oxygen in the photosensitized oxidation of compounds is illustrated in Fig. 7.

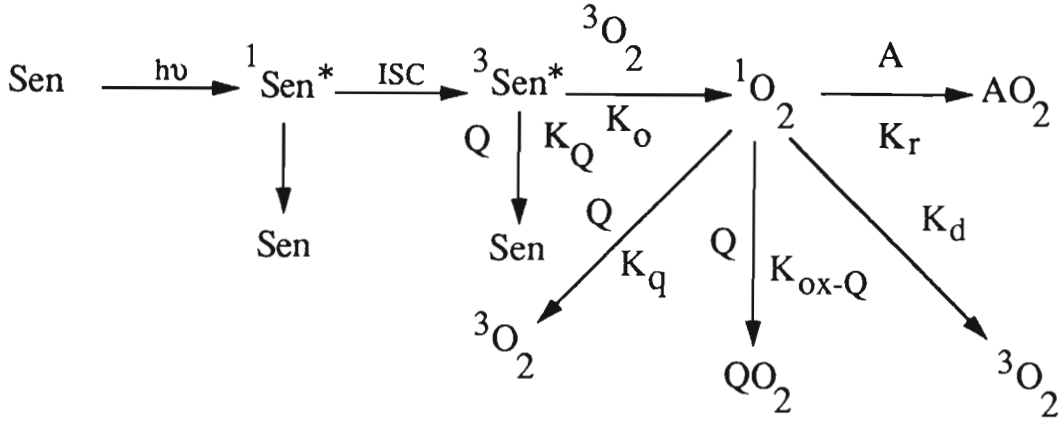


Fig. 7 - Formation and quenching of singlet oxygen

The rate of oxidized compound formation during storage can be expressed as Equation (I) (Fig. 8). The equation shows the formation of excited triplet sensitizer, singlet oxygen, and the oxidized compounds. The symbols used in equation (I) are: AO_2, oxidized compound; A, compound; Q, quencher; K^{-1}, rate of singlet oxygen formation; Ko, reaction rate constant of excited triplet sensitizer and triplet oxygen; K_r, reaction rate constant of compounds with singlet oxygen; K_q, reaction rate constant of physical singlet oxygen quenching by a quencher; K_{ox-Q}, reaction rate constant of chemical singlet oxygen quenching by a quencher; and K_d, the decay rate of singlet oxygen in a solvent.

Equation (I) can be transformed into equation (II) by inverting the Equation (I) (Fig. 8). By plotting the reciprocal of formation rate of the oxidized compounds vs. various concentrations of the compounds at constant concentration of a quencher, a straight line can be acquired. If more than one quencher concentration are used, the quenching mechanisms of the quencher on photosensitized oxidation can be obtained by the intercepts and slopes of those regression lines. As to the mechanism of singlet oxygen quenching only, the y-intercept of regression lines on Fig. 9 is constant and independent of the concentration change of the quencher, but the slopes of the regression lines increase as the concentrations of the quencher increase.

Total quenching rate of singlet oxygen can be obtained by knowing K_r and the slope which can be obtained from the plot in

Fig. 10. With respect to triplet sensitizer quenching only, the intercepts of regression lines of the plot in Fig. 11 are subjected to the quencher concentrations, but the ratio of slope over intercept which is obtained from the equation (II) remains constant (Fig. 12). The K_r can be determined by the slope/intercept and K_d. If triplet sensitizer and singlet oxygen quenchings occur simultaneously, both intercepts and slopes of the regression lines at various quencher concentrations will change accordingly.

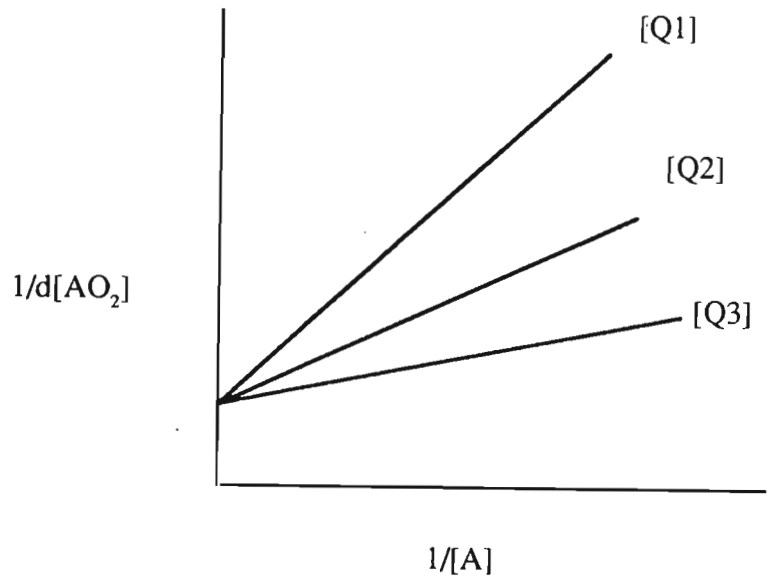
Equation (I)

$$d[AO_2]/dt = K \left\{ \frac{K_o[O_2]}{K_q[Q] + K_o[O_2]} \right\} \left\{ \frac{K_r[A]}{K_r[A] + K_q[Q] + K_{ox\text{-}Q}[Q] + K_d} \right\}$$

Equation (II)

$$\frac{1}{d[AO_2]/dt} = K^{-1} \left\{ \frac{K_q[Q] + K_o[O_2]}{K_o[O_2]} \right\} \left\{ \frac{K_r[A] + (K_q + K_{ox\text{-}Q})[Q] + K_d}{K_r[A]} \right\}$$

Fig. 8 - The formation rate of oxidized compound



$$\frac{1}{d[AO_2]/dt} = K^{-1} \left\{ 1 + \frac{(K_{ox\text{-}Q} + K_q)[Q] + K_d}{K_r[A]} \right\}$$

Fig. 9 - Determination of singlet oxygen quenching mechanism

$$\frac{1}{d[AO2]/dt} = K^{-1}\left\{1 + \frac{(K_{ox\text{-}Q} + K_q)[Q] + K_d}{Kr[A]}\right\}$$

$$S = \frac{K^{-1}\left[(K_{ox\text{-}Q} + K_q)[Q] + K_d\right]}{K_r}$$

$$I = K^{-1}$$

$$S/I = \frac{(K_{ox\text{-}Q} + K_q)[Q] + K_d}{K_r}$$

$$\text{Intercept} = \frac{K_d}{K_r}$$

$$\text{Slope} = \frac{(K_q + K_{ox\text{-}Q})}{K_r}$$

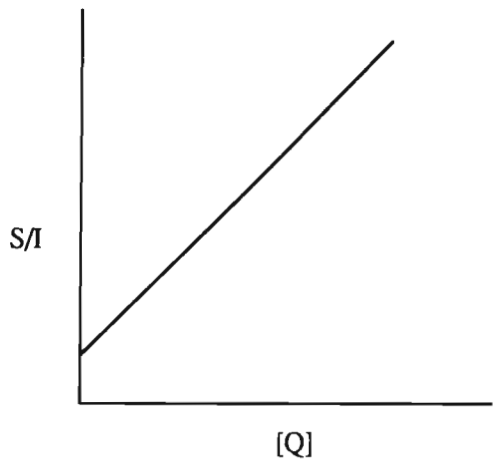
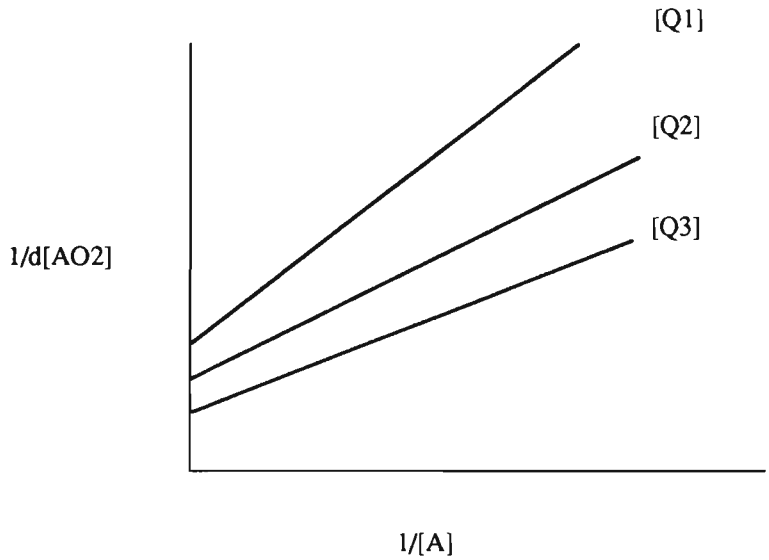
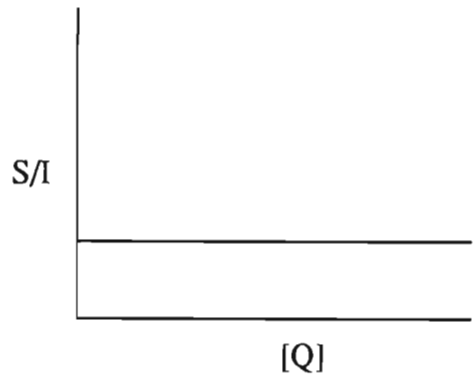


Fig. 10 - The slope/intercept of the plot of Fig. 9 vs. quencher concentrations to determine quenching rate of singlet oxygen



$$\frac{1}{d[AO_2]/dt} = K^{-1}\left\{1 + \frac{K_q[Q]}{K_O[O2]} + \left(\frac{K_d K_q[Q]}{K_O[O_2]K_r} + \frac{K_d}{K_r}\right)\frac{1}{[A]}\right\}$$

Fig. 11 - Determination of triplet sensitizer quenching mechanism



$$\frac{1}{d[AO2]/dt} = K^{-1}\left\{1 + \frac{K_q[Q]}{K_O[O_2]} + \left(\frac{K_d K_q[Q]}{K_O[O_2]K_r} + \frac{K_d}{K_r}\right)\frac{1}{[A]}\right\}$$

$$S = \left(\frac{K_d K_Q[Q]}{K_O[O_2]K_r} + \frac{K_d}{K_r}\right) = K^{-1}\left\{\frac{K_d(K_Q[Q] + K_O[O_2])}{K_O[O_2]K_r}\right\}$$

$$I = \frac{K^{-1}(K_q[Q] + K_O[O_2])}{K_O[O_2]}$$

S: slope

I: intercept

$$S/I = K^{-1}\left\{\frac{K_d(K_q[Q] + K_O[O_2])}{K_O[O_2]K_r}\right\}\left\{\frac{K_O[O_2]}{K^{-1}(K_q[Q] + K_O[O_2])}\right\} = \frac{K_d}{K_r}$$

Fig. 12 - The relationship between slope/intercept of the plot of Fig. 11 and quencher concentrations in triplet sensitizer quenching

8. Roles of Ascorbic Acid in Human Health

Ascorbic acid is an important substance for body welfare due to the following roles (Ensminger et al., 1994).

8.1. Collagen Formation

Collagen is a fibrous protein containing large amounts of proline and hydroxyproline. Ascorbic acid facilitates the formation of hydroxyproline by activating the enzyme prolyl hydroxylase which effects the conversion of proline to hydroxyproline in the formation of collagen. Ascorbic acid also activates the enzyme lysine hydroxylase for the conversion of lysine to hydroxylysine, which is essential amino acid of collagen, by a similar mechanism in the conversion of proline to hydroxyproline.

8.2. Metabolism of Tyrosine, Tryptophan, and Folic Acid

A deficiency of ascorbic acid will cause the accumulation of P-hydroxyphenylpyruvate due to the inactivation of the enzyme P-hydroxyphenylpyruvic acid oxidase in the metabolism of tyrosine. Ascorbic acid can protect the enzyme from inactivation. Ascorbic acid is required to convert tryptophan to 5-hydroxytryptophan which is the first step to form serotonin, which raises blood pressure through vasoconstrictor action. Ascorbic acid is responsible for the conversion of the inactive form of folic acid (folacin) to its active form, folinic acid.

8.3. Iron Utilization

Ascorbic acid facilitates the absorption of ion by converting ferric ion to the more readily absorbed ferrous form. Cereal and infant milks are enriched with ascorbic acid for nutritional fortification and facilitation of the absorption of ion in human body.

8.4. Metabolism of Fats and Lipids

Ascorbic acid acts as cofactor along with Mg^{2+} and ATP to regulate the enzyme adipose tissue lipase, which mobilizes free fatty acids from adipose tissue to meet the energy demands of the body. When the body energy needs have been met, ascorbic acid in combination of Mg^{2+} and ATP can inactivate the adipose tissue lipase. Ascorbic acid is involved in the metabolism of cholesterol. The level of cholesterol increases in the blood serum and liver due to a decrease of conversion rate of cholesterol to bile acids when the ascorbic acid intake is inadequate.

8.5. Antioxidant in the Protection of Vitamin A and E

Ascorbic acid can scavenge oxygen and act as antioxidant to protect vitamin A and E from excessive oxidation.

8.6. Strength of Capillary Walls and Blood Vessels

Ascorbic acid is necessary for maintaining strength of capillary walls and blood vessels. Shortage of ascorbic acid results in

weakened and inelastic capillary walls which may rupture and hemorrhage.

8.7. Removal of Ammonia in the Deamination of Proteins and Peptides

Ascorbic acid can accelerate the deamination of proteins and peptides and the conversion of ammonia to urea for excretion, which has been conjectured to affect aging and longevity.

9. Applications of Ascorbic Acid in Foods

9.1. Use as a Nutrient

Ascorbic acid is considered the most labile of the vitamins in the foods (Edman and Klein, 1982). Ascorbic acid is protected from oxidation in intact plant tissue due to the cellular compartmentation of the tissue. As the tissue is disrupted by mechanical harvesting, the oxidation of ascorbic acid can take place. There are at least four enzymes, ascorbic acid oxidase, polyphenol oxidase, cytochrome oxidase, and peroxidase responsible for the ascorbic oxidation (Mapson, 1970; Krochta and Feinberg, 1975; Erdman and Klein, 1982). Ascorbic acid can be lost during processing and storage. Heat treatment such as blanching, pasteurization and commercial sterilization, or drying can cause a large amount of ascorbic acid loss (Edman and Klein, 1982). Ascorbic acid content of acid fermented products such as cabbage and pickles decreased during tank storage and canning (Pederson, 1960). The 25% of the ascorbic acid content in potato products has been lost after 1 month of storage (Leverton, 1964).

Ascorbic acid loss was also reported during the storage of intermediate moisture apples (Singh et al., 1983). Therefore, ascorbic acid is added to processed foods such as cereal, baked goods, infant milks, cabbage or pickles for supplementing the nutritional loss of ascorbic acid during harvesting, processing and storage to meet nutritional claims.

9.2. Use as a Processing Aid

Most fruits such as apples, apricots, bananas are susceptible to discoloration or browning on cut or injury in the presence of oxygen and enzymes (Bauernfeind, 1982). The discoloration of fruits is due to the activity of phenolases. Ascorbic acid can inhibit the phenolase activity or the enzymatic browning sequence by reducing orthquinone products of the enzyme reaction back to the respective orthophenols (Henshall, 1974; Bauernfeind, 1982). The addition of ascorbic acid to fresh meat can improve the color of the meat as it aids in keeping myoglobin in the oxymyoglobin state (Liu and Watts, 1970). Using ascorbic acid in cooked, cured, and comminuted meat products can reduce curing time, provide more stable and uniform color, and lower nitrite levels of the products (Brown et al., 1974; Bauernfeind, 1982). Nitrosamines such as dimethylnitrosamine are undesirable and toxic compounds, which are formed due to the use of nitrite or nitrate in the meat products. Nitrosamines are formed by the modification of amines (usually secondary and tertiary, but sometimes primary amines) by the nitrosating agent, N_2O_3. N_2O_3 is formed by $2HNO_2 \cdots\cdots> H_2O +$

N_2O_3. Ascorbic acid can prevent the formation of nitrosamines by reacting with HNO_2 to form NO and inhibiting N_2O_3 production (Mirvish et al., 1972). Ascorbic acid is used in breadmaking as flour and dough improver to provide the following advantages: (1) enhanced bread texture and loaf volume, (2) greater elasticity and gas retention to the dough, (3) improved water absorption, (4) no danger of overimprovement or overtreatment, (5) reduced power, or minimal time, or lowered consistency in continuous doughmaking, and (6) storage period of unimproved flour eliminated (Johston and Mauseth, 1969). Ascorbic acid and its esters are used as antioxidants in foods such as fish, margarine, milk, bear, wine, meat to protect the double-bonds containing compounds of foods by scavenging oxygen (Cort, 1982).

10. Factors Affecting Ascorbic Acid Oxidation

Ascorbic acid is an unsaturated lactone which is a strong reducing agent and is easily oxidized. Ascorbic acid is converted to dehydroascorbic acid (DHA) in the presence of oxygen. It is also possible that ascorbic acid can be converted to other products instead of DHA, but the reaction rate of the formation of DHA is greater than the rate of other product formation (Hughes, 1985). DHA can further be hydrolyzed to form diketogulonic acid and then oxalic acid and other species as shown in Fig. 13 (Hughes, 1985; Ensminger et al., 1994).

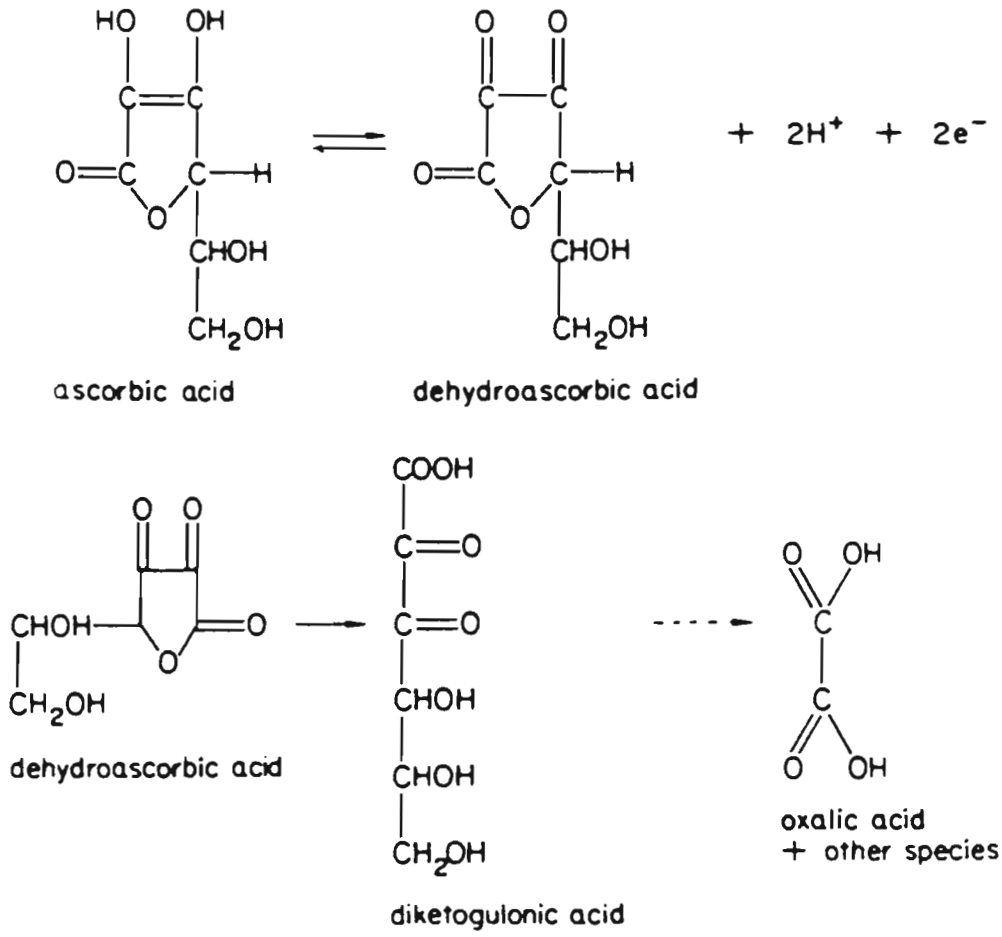


Fig. 13 - Degradation mechanisms of ascorbic acid

Ascorbic acid can degrade to furfural and carbon dioxide. The oxidation of ascorbic acid has been demonstrated as an irreversible reaction, because several factors affect the oxidation reaction and some of those factors are interactive one another (Hughes, 1985).

10.1. pH

Hughes (1985) studied the oxidation of ascorbic acid to dehydroascorbic acid and its degraded product and reported that pH was an important factor to affect the oxidation reaction. Sahbaz and Somer (1993) reported that the reaction rate of ascorbic acid oxidation increased as pH increased from 2.5 to 6.0 in the presence of riboflavin.

10.2. Light

Light has been reported to have influence on the degradation of ascorbic acid. Sattar et al. (1977) reported that ascorbic acid content in pure solution did not have significant change when ascorbic acid was exposed to fluorescent light. However, ascorbic acid content decreased remarkably in the presence of riboflavin during 3 hr light storage. Photosensitized decomposition of ascorbic acid in the presence of riboflavin was confirmed by Sahbaz and Somer (1993).

10.3. Metal Ions

Heavy metals, especially those with two or more valences, have been known to have catalytic ability. A series of metal ions, Cu^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+}, and Fe^{3+} have been studied for the catalytic effect on the oxidation of ascorbic acids. Cu^{2+}, and Fe^{3+} both accelerated the decomposition of ascorbic acid under dark or light conditions. Catalytic activity of Cu^{2+} was higher than that of Fe^{3+}. Pb^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+} had no influence on the decomposition of ascorbic acid (Sattar et al., 1977). Chelating agents such as EDTA, poly-L-histidine, and citrate were reported to decrease the decomposition rate of ascorbic acid by complexing Cu^{2+} to modify its catalytic activity (Pecht et al., 1967; Sahbaz and Somer, 1993).

10.4. Oxygen

Johnson and Toledo (1975) studied the oxidation of ascorbic acid in orange juice concentrate and found that an increase of oxygen concentration in the headspace increased the rate of oxidation. Blaug and Hajratwala (1972) reported that increased ascorbic acid concentration did not increase the rate of oxidation with constant oxygen concentration suggesting that oxygen was limiting. Waletzko and Labuza (1976) investigated the destruction of ascorbic acid in intermediate moisture food packaged with air and N_2/H_2 atmosphere, and reported that ascorbic acid was more stable in the N_2/H_2 atmosphere than in the air.

10.5. Temperature

Eison-Perchonok and Downes (1982) investigated the effects of temperature and dissolved oxygen concentration on the oxidation of ascorbic acid. The temperature and the headspace oxygen concentration ranged from 30 to 55°C and 10 to 21%, respectively. The decomposition rate of ascorbic acid was highest at the combination of 55°C and 15 % headspace oxygen. The degradation of ascorbic acid at high temperature (61-105°C) was studied by Laing et al. (1978). They reported that the degradation rate of ascorbic acid increased as the temperature increased from 61-92°C, but the rate decreased as the temperature increased from 95-105°C. They concluded that the solubility of oxygen in aqueous phase was the major reason, because the solubility of oxygen in pure water decreased with increasing temperature up to 100°C, where it approaches zero. The degradation of ascorbic acid at high temperature still occurred in the absence of oxygen, but the oxidative degradation tended to predominate.

11. Ascorbic Acid Analysis of Foods

Many factors and different food systems can affect the oxidative stability of ascorbic acids. Different analytical methods have to be applied for the measurement of ascorbic acid in different food systems. The 2, 6-dichloroindophenol titrimetric method is a common official method (AOAC, 1990). Dodson et al. (1992) commented that titrimetric and colorimetric

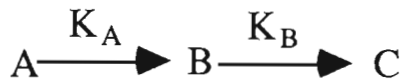
methods lack the specificity and are subject to matrix interference. The microfluorometric method is specific for total ascorbic acid, which is applicable for products rich in ascorbic acid. The products with low levels of ascorbic acid require a large amount of sample which increases the possibility of matrix interference. The investigators first oxidized ascorbic acid to dehydroascorbic acid with Norit and followed by reaction with o-phenylenediamine to form a fluorescent derivative. The derivative was then separated by liquid chromatography and detected by fluorometry. They reported that the recoveries of ascorbic acid were in the range of 90-108% and the results were in excellent agreement with the AOAC fluorometric method. Ashoor et al. (1984) used liquid chromatography in ascorbic acid analysis which can eliminate the interference of pigments of foods and obtained favorable results compared to AOAC method. They also studied the wavelength for ascorbic absorption, and found 245 nm was the maximum response for the detector under their conditions. Ascorbic acid and dehydroascorbic acid consist of total vitamin C content of foods. The 2, 6-dichloroindophenol titrimetric method detects the reduced form of ascorbic acid only. Wilmalasiri and Wills (1983) were able to separate ascorbic acid from dehydroascorbic acid by high-performance liquid chromatography and detected them at 254 and 214 nm, respectively. When the concentration of dehydroascorbic acid is low, the detection at 210 nm is not sensitive enough to accurately measure its content due to inference of solvent at low wavelength (Dodson et al., 1992).

Kim (1989) used dithiothreitol to reduce dehydroascorbic acid to ascorbic acid and determined the total vitamin C by ion exclusion chromatography with electrochemical detection. He reported the method was specific for ascorbic acid and the results were agreed with expected values. The time for the analysis was 10 min and the detectable concentration for sensitivity was 0.1 ng. Tsumura et al. (1993) reported a rapid enzymatic assay for ascorbic acid in various foods. They first reduced the dehydroascorbic acid indigenously present in extract of foods by 2-mercaptoethanol and recorded the initial value of absorbance of ascorbic acid at 265 nm. Then guaiacol peroxidase and hydrogen peroxide were added to oxidize ascorbic acid to dehydroascorbic acid during which the absorbance decreased as the oxidation proceeded. After the reaction was completed, they calculated total decrease of absorbance which were converted and expressed as total vitamin C content.

12. Kinetics of Ascorbic Acid Degradation

Reaction order has been of great interest to investigators with respect to kinetics of ascorbic acid oxidation. Waletzko and Labuza (1976) reported that the degradation of ascorbic acid in an intermediate moisture food followed a first order reaction either in the air or in a N2/H2 atmosphere. Eison-Perchonok and Downes (1982) also reported a first order reaction of ascorbic acid oxidation. Singh et al. (1983) studied the degradation of ascorbic acid of intermediate moisture apples and confirmed a first order

reaction at temperature ranging from 25 to 55°C and water activity from 0.62 to 0.89. While Karel and Nickerson (1964) observed a zero order reaction of ascorbic acid degradation in their study of dehydrated orange juice. Laign et al. (1978) used high concentration of ascorbic acid (400 mg/100g solids) to study high temperature (61-92°C) effects on ascorbic acid decomposition and reported a zero order reaction. They explained that the zero order reaction may be due to high initial concentration of ascorbic acid necessary for high temperature studies. As a result, the percentage change of ascorbic acid was relatively low. The reaction order of ascorbic acid oxidation may shift depending on the conditions such as the concentration of dissolved oxygen and the presence of catalysts (Sakai et al., 1987). Deng et al. (1978) studied ascorbic acid degradation in fish flesh and found the first order kinetics in dark or mixed flesh, but zero order in light color flesh. They suggested that dark flesh contained higher lipid oxidation catalysts such as iron than did the light flesh and thus accelerated the degradation of ascorbic acid. Sakai et al. (1987) proposed a model to rationalize zero or first order kinetics for ascorbic acid degradation. The mode is composed of consecutive reactions as the following:



Where A is ascorbic acid; B, an intermediate; C, an oxidized product; K_A, zero-order rate constant with respect to ascorbic acid concentration; K_B, first-order rate constant with respect to intermediate concentration. When $K_A > K_B[Ao]$, the reaction is a first-order reaction. When $K_A < K_B[Ao]$ the reaction is a zero-order reaction, where Ao is the initial concentration of ascorbic acid.

III. METERIALS AND METHODS

1. Materials

Soybean oil was supplied by Karlsham Co. (Columbus, OH). FD&C Red No. 40, Red No. 3, Yellow No. 5, Yellow No. 6, Green No. 3, Blue No. 1, and Blue No. 2 were obtained from Warner Jenkison Co. (St. Louis, MO). Methylene blue, Rose bengal and Eosin B were obtained from Eastman Kodak Co. (Rochester, NY). β-Carotene was purchased from Fluka Chemical Co. (Ronkonkoma, NY). α-Tocopherol was obtained from Hoffmann-Roche Inc. (Nutley, NJ). Dibasic sodium phosphate, citric acid, and L-ascorbic acid were purchased from Sigma Chemical Co (St. Louis, MO). Acetone was purchased from Mallinckrodt (Paris, KY).

2. Sample Preparation for the Reproducibility of Gas Chromatographic Headspace Oxygen Analysis

The 0.02 M soybean oil containing 20 ppm Red No. 3 in acetone was prepared in 6 replicates and stored under light for 1 hr at 25°C. The headspace oxygen of the sample bottles were analyzed by gas chromatography. The data was statistically analyzed for coefficient of variation.

3. Effects of Synthetic Colorants on the Oxidation of Soybean Oil during Storage

3.1. Sample Preparation

Rose bengal, Eosin B, methylene blue, FD&C Red No. 40, Red No. 3, Yellow No. 5, Yellow No. 6, Green No. 3, Blue No. 1, and Blue No. 2 were used. A powdered colorant was dissolved in distilled water to obtain the concentration of 500, 2000, 10000, and 20000 ppm. These solutions were used as colorant stock solutions to obtain the experimental samples of 5, 20, 100, and 200 ppm colorant in acetone throughout the experiments. The concentrations of 5, 20, 100, and 200 ppm colorants in the acetone containing 0.03 M soybean oil were prepared by adding 1 ml of each colorant stock solution to each 99 ml acetone containing 2.78 ml soybean oil. One ml distilled water was used to replace 1 ml color solution as a control sample. Twenty-five ml of each sample solution was transferred to 40 ml glass serum bottles. The sample bottles were sealed air-tight with Teflon-lined rubber septa and aluminum caps (Supelco Inc., Bellefonte, PA). All of the samples under each treatment were prepared in duplicate or triplicate in some cases.

3.2. Storage of Sample Bottles

3.2.1. Light Box for Sample Storage

A light box was especially designed for storing samples as shown in Fig. 14. The inside of a wooden box (70 cm x 50 cm x 60 cm) was lined with mirrors to provide uniform light intensity to

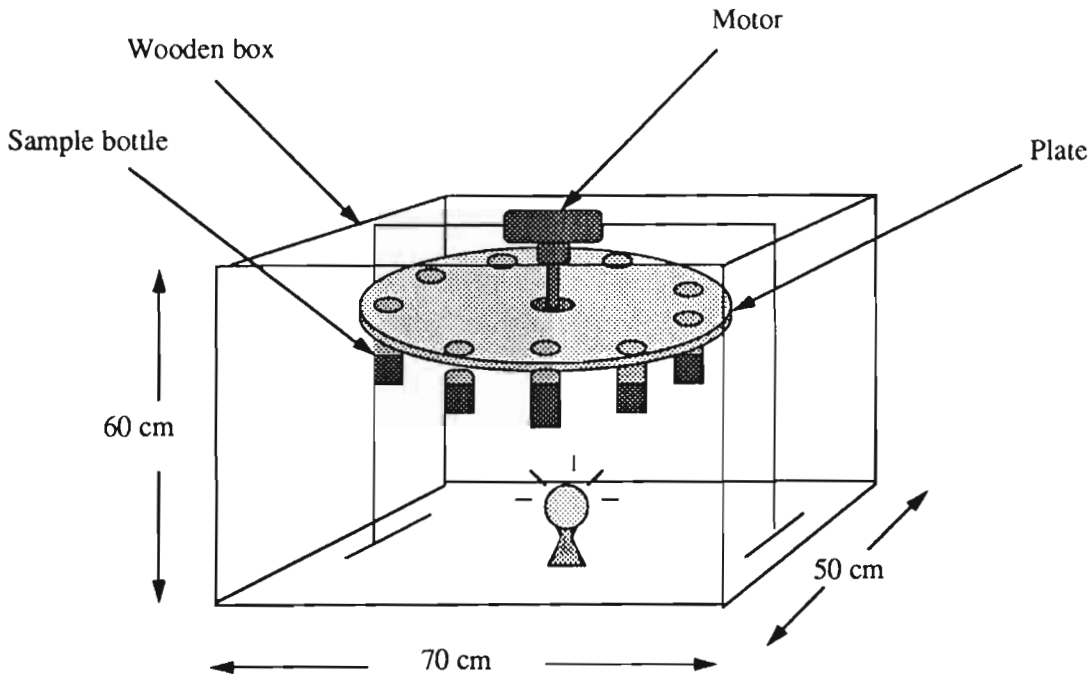


Fig. 14 - Diagram of light box for storage of samples

samples during storage. A circular plastic plate (id, 35 cm) was designed to load sample bottles. The plate loaded with sample bottles was driven by a motor (Talboys Engineering Co., Emerson, NJ) to provide uniform light intensity to every sample bottle. The speed of the motor was controlled at 60 rpm by a variable autotransformer (Staco Energy Products Co., Dayton, OH). A 200 W tungsten lamp (GE, Cleveland, OH) as a light source was placed 15 cm under the center of the plate. The light intensity of the sample bottles was about 4,000 lux.

3.2.2. Sample Storage Conditions

The sample bottles were divided into two groups. One group in duplicate was stored in the dark environment as a control. The other group in duplicate was stored in the light box for 1, 2 or 4 hours. The samples containing no colorant was used as control samples under light storage. The sample bottles containing the synthetic colorants were stored under light and dark in a 25°C temperature-controlled chamber.

3.3. Headspace Oxygen Analysis

The effects of synthetic colorants on the oxidation of soybean oil under light and dark were evaluated by measuring the depletion of headspace oxygen of sample bottles during storage. The headspace oxygen of sample bottles was analyzed by a gas chromatograph (HP 5890, Hewlett Packard, Avondale, PA) equipped with a stainless steel molecular sieve column (13X, 80/100,

Alltech, Deerfield, IL) and a thermal conductivity detector (Hewlett Packard, Avondale, PA). High purity helium (99.99%) was used as carrier and auxiliary gas with a flow rate of 30 ml/min for both. An isothermal oven temperature of 50°C was used. One hundred µl headspace gas was injected onto the column. The injection port and detector temperatures were 120°C and 150°C, respectively. One hundred µl air from the laboratory was injected onto the GC to use as reference gas chromatographic oxygen peak to establish daily gas chromatographic variation. The daily changes of gas chromatographic headspace oxygen peak due to analytical variations can be corrected by using the oxygen peak of air. Gas chromatographic peak area of oxygen in 100 µl of headspace gas was quantified by using a HP 3390A electronic integrator and expressed as electronic counts. The electronic counts of oxygen content in 100 µl headspace gas were converted to µmole oxygen in 1 ml headspace gas. One hundred µl of air contained 0.935 µmole oxygen (Parker, 1982). The electronic counts of 0.935 µmole oxygen were obtained by injecting 100 µl air into the GC and electronic counts of 1 µmole oxygen were calculated.

4. Sample Preparation and Analyses for the Quenching Mechanisms and Kinetics of α-Tocopherol on the Red No. 3 Photosensitized Oxidation of Soybean Oil

The 4 x 3 x 2 factorial design at 0.01, 0.02, 0.03, and 0.04 M soybean oil; 0, 9.29 $x10^{-5}$, or 1.86 $x10^{-4}$ M α-tocopherol and 20 ppm Red No. 3 in acetone were prepared. Twenty-five ml of each sample solution were transferred to 40 ml glass serum bottles. The sample bottles were sealed air-tight with Teflon-lined rubber septa and aluminum caps (Supelco Inc., Bellefonte, PA). The samples were prepared in duplicate. The prepared sample bottles were divided into two groups. One group was stored in the dark environment as control, and the other group was stored in the light box for 1 hour. The light box was placed in a temperature-controlled chamber to maintain the temperature of the light box of 25°C. The headspace oxygen depletion of sample bottles was studied by using steady-state kinetics to determine the quenching mechanisms and reaction rates of α-tocopherol on soybean oil oxidation under light in the presence of Red No. 3 (Foote, 1979; Lee and Min, 1990).

5. Sample Preparation and Analyses for the Quenching Mechanisms and Kinetics of β-Carotene on the Red No. 3 Photosensitized Oxidation of Soybean Oil

The 4 x 5 x 2 factorial design at 0.01, 0.02, 0.03, and 0.04 M soybean oil; 0, 9.3 $x10^{-7}$, or 1.86 $x10^{-6}$, 3.72 $x10^{-6}$, 7.44 $x10^{-6}$ M β-carotene and 20 ppm Red No. 3 in acetone was prepared.

Twenty-five ml of each sample solution were transferred to 40 ml glass serum bottles. The sample bottles were sealed air-tight with Teflon-lined rubber septa and aluminum caps (Supelco Inc., Bellefonte, PA). The samples were prepared in duplicate. The prepared sample bottles were divided into two groups. One group was stored in the dark environment as control, and the other group was stored in the light box for 1 hour. The light box was placed in a temperature-controlled chamber to maintain the inside temperature of the light box of 25°C. The headspace oxygen depletion of sample bottles was measured by gas chromatography. The results were studied by using steady-state kinetics to determine the quenching mechanisms and reaction rates of β-carotene on soybean oil oxidation under light in the presence of Red No. 3 (Foote, 1979; Lee and Min, 1990).

6. Effects of Red No. 3 on the Oxidation of Ascorbic Acid

6.1. Preparation of Buffer Solution

Crystalline citric acid and dibasic sodium phosphate were dissolved in distilled water to prepare 0.1 M and 0.2 M stock solutions. The buffer solution of pH 4 or 5.6 or 7 was prepared by the proper combination of the stock solutions (Gerhardt, 1981). The buffer solution bottles were sealed with paraffin film and stored in refrigerator at 4°C until use.

6.2. Sample Preparation for the Spectrophotometric Determination of Ascorbic Acid

One hundred mg crystalline L-ascorbic acid were dissolved in 10 ml distilled water to prepare the 10,000 ppm stock solution. The ascorbic acid solutions of 20, 40, 60, 80 and 100 ppm ascorbic acid were prepared by diluting the stock solution in pH 7 buffer solution. The ascorbic acid solutions were wrapped with aluminum foil and placed in iced water to prevent the effects of light and temperature on the ascorbic acid prior to analyses. Ascorbic acid content was measured by a spectrophotometer, Spectronic® 1201, (Milton Roy Co., Linden, NJ) equipped with a UV detector at 265 nm (Tsumura et al., 1993). The correlation of absorbance and ascorbic acid content was obtained by linear regression analysis.

6.3. Sample Preparation for the Effect of pH on the Color Stability of Red No. 3

The 40 ppm Red No. 3 was prepared in pH 4, 5.6, or 7 solution. Twenty-five ml of the sample solution were transferred to 40 ml serum bottles and sealed air-tight with septa and aluminum caps. The sample bottles were prepared in triplicate. The samples were stored under light and dark for 1 hr at 25°C. The Red No. 3 in the sample bottle was determined by a spectrophotometer at 525 nm (Rosenthal, 1985).

6.4. Sample Preparation for the Effects of Red No. 3 on the Oxidation of Ascorbic Acid

Fifty, 100, 150 and 200 ppm ascorbic acid were prepared by diluting the 10,000 ppm stock solution in the pH 4, 5.6 or 7 buffer solution containing 40 ppm Red No. 3. Twenty-five ml the mixed solution were transferred to a 40 ml serum bottle and sealed air-tight with an aluminum cap and a Teflon septum. The samples without Red No. 3 were used as control. The sample bottles were stored under dark or under light for 1 hr at 25 °C. The ascorbic acid content of sample bottles was determined by a spectrophotometer at 265 nm and gas chromatographic headspace analysis.

7. Statistical Analysis

The data were analyzed statistically by ANOVA and t-test using computer programs, Cricket Graph™ (Cricket Software, Malvern, PA) and StatView™ (BrainPower Inc., Calabasa, CA).

IV. RESULTS AND DISCUSSION

1. Reproducibility of Headspace Oxygen Analysis by Gas Chromatography

The headspace oxygen of the sample containing 20 ppm Red No. 3 and 0.02 M soybean oil in acetone under light for 1 hr were analyzed 6 times by gas chromatography to determine the reproducibility of the headspace oxygen analysis. The results are shown in Table 6. The coefficient of variation was 0.55%, which indicated good reproducibility of gas chromatographic headspace oxygen analysis. The coefficient of variation of the headspace analysis in the preliminary study was about 8%. The reproducibility was improved by (1) maintaining proper concentration of oil and Red No. 3 in acetone to maintain stable solution during storage, (2) lining the inside walls of storage box with mirrors for better reflection of light, (3) designing a motor-driven plate to provide uniform light intensity to samples under light storage.

Table 6 - Coefficient of variation for the gas chromatographic headspace oxygen analysis of sample bottles

Analysis	O_2 % (X)	X-A	$(X-A)^2$
1	16.55	0.07	0.0049
2	16.58	0.10	0.0100
3	16.39	-0.09	0.0081
4	16.47	0.01	0.0001
5	16.35	-0.13	0.0169
6	16.51	0.03	0.0009
	A=16.48		$\Sigma (X-A)^2 = 0.0409$

A= Average value of 6 replicate analyses

$$\text{Variance } (S^2) = \frac{\Sigma (X-A)^2}{n-1} = \frac{0.0409}{5} = 0.00818$$

Standard deviation (S) = 0.0904

$$\text{Coefficient of variation (CV)} = \frac{100 \times S}{A} = \frac{100 \times 0.0904}{16.48} = 0.55\ \%$$

2. Model Sample for Studying Lipid Oxidation in the Presence of Water Soluble Synthetic Colorants

An oil/water in acetone solution was developed to study the photosensitized lipid oxidation in the presence of water soluble synthetic colorants under light. The sample solution provides a soluble solution of hydrophilic colorants and hydrophobic oil in acetone. The proper ratio of oil / water in acetone is crucial to maintain good solution stability during storage. The ratios of oil / water / acetone used were 1/1/98, 2/1/97, 3/1/96, and 4/1/95 (v/v/v). The oil-water-acetone system provided a stable solution during storage.

The effects of 100 ppm FD&C Red No.3, Eosin B, rose bengal, and methylene blue on the soybean oil oxidation under light were studied. The samples containing the 100 ppm colorants and 0.03 M oil in acetone were stored under light and dark for 2 hr at 25°C. The headspace oxygen depletion of samples is shown in Table 7. The headspace oxygen of samples with colorants stored under light decreased while the headspace oxygen of the samples without 100 ppm colorants under light or the samples with 100 ppm colorants under dark did not decrease. The prooxidant effects of the colorants under light must be due to the photosensitizing effects to form singlet oxygen by a triplet colorant - triplet oxygen annihilation mechanism. The reaction rate of linoleic acid with singlet oxygen is 1500 times greater than that with ordinary triple oxygen (Rawls and Van Santen, 1970). The results in Table 7 indicated that FD&C Red No.3, Eosin B, rose bengal, and

methylene blue accelerated the oxidation of soybean oil under light. However, these compounds did not have any effect on the oxidation of soybean oil under dark. The prooxidant activity of the colorants under light was in the order of methylene blue > rose bengal > Red No. 3 > Eosin B. The absorption spectra of the colorants ranged from 435 to 750 nm, which indicated no special visible spectrum required for a colorant to be a photosensitizer. The oil-colorant-acetone model system and headspace oxygen analysis can be used to study the photosensitizing effect of water soluble colorants on lipid oxidation.

Table 7 - Effects of 100 ppm Red No.3, Eosin B, rose bengal, and methylene blue on the headspace oxygen depletion of the oil sample bottles under light for 2 hr

	Headspace oxygen depletion* (%)			
	Under light		Under dark	
	0 ppm	100 ppm	0	100 ppm
Eosin B	0	8	0	0
Red No. 3	0	63	0	0
Rose bengal	0	69	0	0
Methylene blue	0	77	0	0

* Oxygen content of air is expressed as 100%

3. Effect of Oil Concentrations on the Headspace Oxygen Depletion of Red No. 3 Photosensitized Oxidation of Soybean Oil

The headspace oxygen contents of samples containing 20 ppm Red No. 3 and 0.01, 0.02, 0.03, and 0.04 M soybean oil were analyzed by gas chromatography in triplicate after 1 hr storage under light. The gas chromatograms of headspace oxygen in the Red No. 3 photosensitized soybean oil are shown in Fig. 15. The chromatograms clearly show a well separated oxygen peak. The effect of soybean oil concentration on the headspace oxygen content of samples in the presence of 20 ppm Red No. 3 under light storage for 1 hr is shown in Table 8. The results indicated that as the oil concentration increased, the headspace oxygen content decreased significantly at $\alpha = 0.05$. However, the headspace oxygen contents of 0.01, 0.02, 0.03, and 0.04 M soybean oil in the absence of Red No. 3 under light or in the presence 20 ppm Red No. 3 under dark for 1 hr were essentially the same of about 21.65 %.

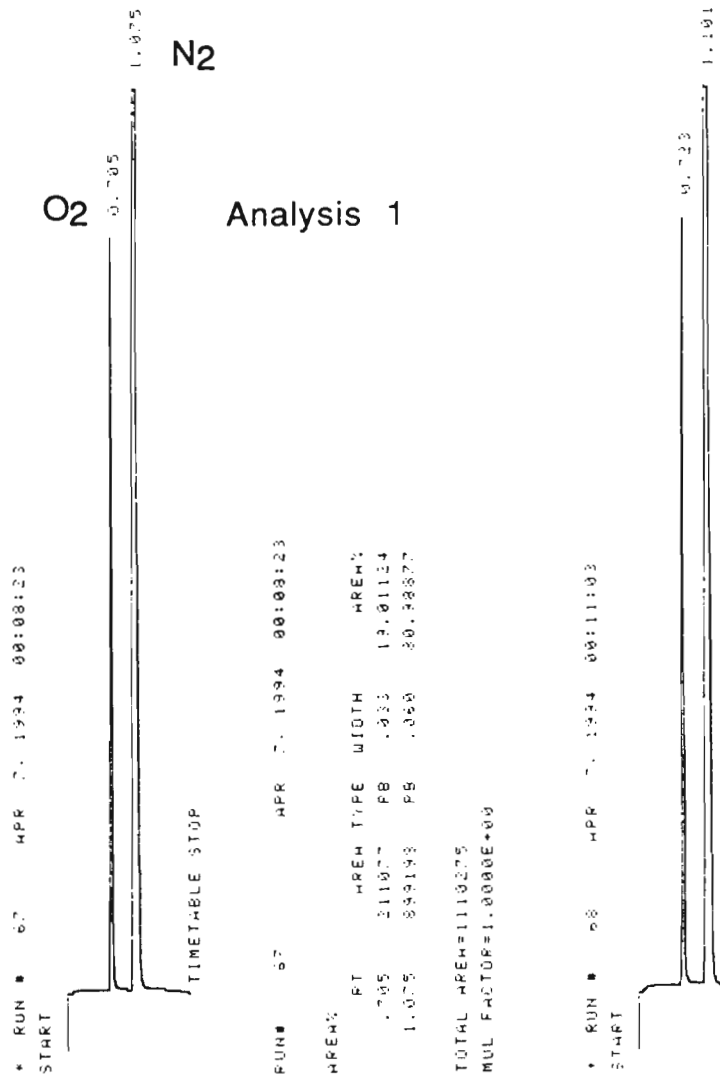


Fig. 15 - Gas chromatograms of the headspace oxygen content of sample bottle containing 0.01 M soybean oil and 20 ppm Red No. 3 in acetone under light for 1 hr

Table 8 - Effect of soybean oil concentrations on the headspace oxygen depletion of sample bottles containing 20 ppm Red No. 3 in acetone under light for 1 hr

	Headspace oxygen* (%)				
Soybean oil	Trial 1	Trial 2	Trial 3	Mean**	CV (%)
0.01M	18.94	19.01	19.00	18.99[a]	0.17
0.02M	16.55	16.58	16.39	16.50[b]	0.58
0.03M	14.59	14.66	14.51	14.59[c]	0.52
0.04M	12.94	13.05	12.81	12.93[d]	0.93

* Oxygen content of air was assumed to be 21.65% under the experimental conditions.

** Mean values in the same column with different superscript letters are significant at $\alpha = 0.05$.

4. Effects of FD&C Colorants on the Oxidation of Soybean Oil under Light

The effects of FD&C Red No. 40, Red No. 3, Yellow No. 5, Yellow No. 6, Green No. 3, Blue No. 1, and Blue No. 2 on the oxidation of soybean oil under light storage were studied. The samples containing 0, 5, 20, 100, and 200 ppm colorant and 0.03 M soybean oil in acetone were prepared in duplicate and stored under light for 1, 2, and 4 hr. The effects of colorant concentrations and storage time on the headspace oxygen contents are shown in Table 9. The headspace oxygen of samples containing Red No. 3

decreased as the storage period increased under light, but the headspace oxygen of the sample in the absence of Red No. 3 under light did not change. The headspace oxygen of sample bottles containing the FD&C Red No. 40, Yellow No. 5, Yellow No. 6, Green No. 3, Blue No. 1, and Blue No. 2 did not decrease. Red No. 3 acted as photosensitizer to accelerate the oxidation of soybean oil under light by forming singlet oxygen through excited triplet Red No. 3 and triplet oxygen annihilation mechanism. As the concentration of Red No. 3 increased, the headspace oxygen content decreased. The headspace oxygen of the sample containing 5 ppm Red No. 3 decreased from 21.28 to 16.89 % under light for 1 hr. As the concentration of Red No. 3 increased from 5 to 200 ppm the headspace oxygen content decreased from 16.89 to 11.20%. The headspace oxygen depletion between the samples containing 100 or 200 ppm Red No. 3 after 2 and 4 hr under light did not show a significant difference at 5 % level. Red No. 3 at the concentration of 100 or 200 ppm may have a filtering effect to reduce the light intensity and the reduced light intensity may reduce the formation of excited Red No. 3 to produce singlet oxygen. The excited triplet Red No. 3 molecules may not be increased proportionally to the concentration of singlet Red No. 3 molecules in the sample due to the light filtering effect as well as low concentration of remaining headspace oxygen. Excited triplet Red No. 3 molecules are responsible for the formation of singlet oxygen from triplet oxygen by triplet-triplet annihilation.

The rapid singlet oxygen reaction with soybean oil will decrease the headspace oxygen rapidly.

Table 9 - Effects of FD&C colorants on the headspace oxygen of soybean oil under light storage

	Headspace oxygen (%)*		
FD&C colorant or control (ppm)	1	2	4 (hr)
Blue No. 1			
0	21.28	21.01	20.87
5	21.25	20.92	20.10
20	21.27	21.32	21.08
100	21.28	21.19	20.93
200	21.29	21.33	21.10
Blue No. 2			
0	21.28	21.01	20.87
5	21.25	21.38	21.16
20	21.33	21.46	21.24
100	21.34	21.43	21.51
200	21.29	21.43	21.50
Red No. 3**			
0	21.28[a]	21.01[a]	20.87[a]
5	16.89[b]	12.25[b]	6.30[b]
20	14.39[c]	8.57[c]	5.64[c]
100	12.24[d]	6.87[d]	4.64[d]
200	11.20[e]	6.85[d]	4.58[d]

Green No. 3			
0	21.28	21.01	20.87
5	21.35	21.30	20.52
20	21.33	20.97	20.40
100	21.14	21.00	21.03
200	21.04	20.68	20.01
Yellow No. 5			
0	21.28	21.01	20.87
5	21.16	20.51	20.38
20	21.36	21.38	21.19
100	21.40	21.42	21.37
200	21.24	21.20	21.00
Yellow No. 6			
0	21.28	21.01	20.87
5	21.33	21.40	21.02
20	21.35	21.43	20.67
100	21.25	21.37	21.33
200	21.34	21.44	21.28
Red No. 40			
0	21.28	21.01	20.87
5	21.24	21.33	21.24
20	21.30	21.32	21.07
100	21.29	21.35	21.17
200	21.33	21.40	21.29

* Mean value of duplicate analyses.

* * The same superscript letters in the same Red No. 3 column indicate no significant difference (α=0.05).

5. Quenching Mechanisms and Kinetics of α-Tocopherol on the Red No. 3 Photosensitized Oxidation of Soybean Oil

The effects of 0, 9.29 x 10^{-5} and 1.86 x 10^{-4} M α-tocopherol on 20 ppm Red No. 3 photosensitized oxidation of soybean oil under light for 1 hr are shown in Table 10.

Table 10 - Effects of soybean oil and α-tocopherol concentrations on the headspace oxygen depletion of samples containing 20 ppm Red No. 3

Headspace oxygen depletion (μmole O_2/ml headspace)*			
	α-Tocopherol (M)		
Soybean oil	0	9.29×10^{-5}	1.86×10^{-4}
0.01M	$1.10^{a}{}_{a}$**	$0.95^{a}{}_{b}$	$0.85^{a}{}_{c}$
0.02M	$2.22^{b}{}_{a}$	$1.85^{b}{}_{b}$	$1.66^{b}{}_{c}$
0.03M	$2.77^{c}{}_{a}$	$2.63^{c}{}_{a}$	$2.36^{c}{}_{b}$
0.04M	$4.15^{d}{}_{a}$	$3.57^{d}{}_{b}$	$3.13^{d}{}_{c}$

* Mean of duplicate analyses.

** The same superscript letters in the same column or the same subscript letters in the same row are not significantly different at α=0.05.

The headspace oxygen depletion of soybean oil in the absence of α-tocopherol was greater than that in the presence of 9.29×10^{-5} or 1.86×10^{-4} M α-tocopherol. As the concentration of α-tocopherol increased, the headspace oxygen depletion decreased. The headspace oxygen of soybean oils containing 20 ppm Red No. 3 and 0, 9.29×10^{-5} and 1.86×10^{-4} M α-tocopherol did not decrease under dark for 1 hr. The headspace oxygen of soybean oils containing 9.29×10^{-5} or 1.86×10^{-4} M α-tocopherol and no 20 ppm Red No. 3 under light for 1 hr did not decrease. The antioxidant effect of α-tocopherol on the Red No. 3 photosensitized oxidation of soybean oil may be due to the quenching of singlet oxygen and/or the excited triplet Red No. 3 or both. α-Tocopherol has been reported to quench the singlet oxygen only in the chlorophyll photosensitized oxidation of soybean oil (Jung et al., 1991). However, whether α-tocopherol quenches Red No. 3 or not has not been studied.

The α-tocopherol quenching mechanisms and rates for singlet oxygen or triplet sensitizer as well as the singlet oxygen reaction rate with soybean oil were studied by using steady-state kinetics (Foote, 1979; Jung et al., 1991). The kinetic equation for the formation rate of oxidized soybean oil in the presence of Red No. 3 and α-tocopherol is

$$\{d[AO_2]/dt\}^{-1} = K^{-1}\{(Ko[O_2] + KQ[Q])/Ko[O_2]\}\{[Kr[A] + (Kox\text{-}Q + Kq)[Q] + Kd]/Kr[A]\}$$

Where AO_2, oxidized compound; A, compound; Q, quencher; K^{-1}, rate of singlet oxygen formation; Ko, reaction rate constant of triplet sensitizer and triplet oxygen; Kr, reaction rate constant of the compound with singlet oxygen; Kq, reaction rate constant of physical singlet oxygen quenching by a quencher; Kox-Q, reaction rate constant of chemical singlet oxygen quenching by a quencher; and Kd, the decay rate of singlet oxygen in a solvent. When there is only singlet oxygen quenching, the above equation can be reduced to the following:

$$\{d[AO_2]/dt\}^{-1} = K^{-1}\{1 + [(Kox\text{-}Q + Kq)[Q] + Kd]/Kr[A]\}$$

The plots of $[AO_2]^{-1}$ vs. $[A]^{-1}$ at various [Q] give a constant y-intercept which is K^{-1}. The constant y-intercept indicates that the quencher quenched singlet oxygen only. The slope of the equation is $K^{-1}\{Kd/Kr + (Kox\text{-}Q + Kq)[Q]/Kr\}$. The slope (S) divided by the intercept (I), S/I, is $\{Kd/Kr + (Kox\text{-}Q + Kq)[Q]/Kr\}$. The plot of S/I vs. various [Q] gives a new intercept of the plot, which is Kd/Kr and a new slope which is (Kox-Q + Kq)/Kr. If Kd is known, Kr can be calculated. The total singlet oxygen quenching rate (Kox-Q + Kq) can be obtained when Kr is known. Table 11 shows the intercepts, slopes, and slope/intercept from the plots of $[AO_2]^{-1}$ vs. $[A]^{-1}$ (Fig. 16). The values of Table 11 can be used to determine the quenching mechanism of α-tocopherol on the Red No. 3 photosensitized oxidation of soybean oil. The y-intercept of the plots of $[AO_2]^{-1}$ vs. $[A]^{-1}$ at 0, 9.29×10^{-5}, and 1.86×10^{-4} M α-

tocopherol are the same but the slopes are different. The constant y-intercept at different concentrations of α-tocopherol indicated that the quenching mechanism of α-tocopherol on Red No. 3 photosensitized oxidation of soybean oil was due to singlet oxygen quenching only. α-Tocopherol did not quench triplet Red No. 3.

Table 11 - The intercepts and slopes of the plots of $[AO_2]^{-1}$ vs. $[A]^{-1}$ of Fig. 16 to determine the quenching mechanism and rate of α-tocopherol on the Red No. 3 photosensitized oxidation of soybean oil

α-Tocopherol (M)	Intercept (ml headspace/μmole O_2)	Slope (M-ml headspace/μmole O_2)	S/I (M)
0	3.7×10^{-2}	8.6×10^{-3}	0.23
9.29×10^{-5}	3.7×10^{-2}	1.0×10^{-2}	0.27
1.86×10^{-4}	3.6×10^{-2}	1.15×10^{-2}	0.32

The plot of S/I vs. 0, 9.29×10^{-5} and 1.86×10^{-4} M α-tocopherol (Fig. 17) has an intercept of 0.23 M and a slope of 485. Since Kd/Kr is equal to 0.23 M and Kd is 1.96×10^4 Sec^{-1} in acetone (Gorman and Rogers, 1989), Kr is 8.5×10^4 $M^{-1}Sec^{-1}$. The total quenching rate, (Kq + Kox-Q), of tocopherol on singlet oxygen is 4.1×10^7 $M^{-1}Sec^{-1}$. The rate constants for singlet oxygen quenching by α-tocopherol have been reported as 2.6×10^7 $M^{-1}Sec^{-1}$

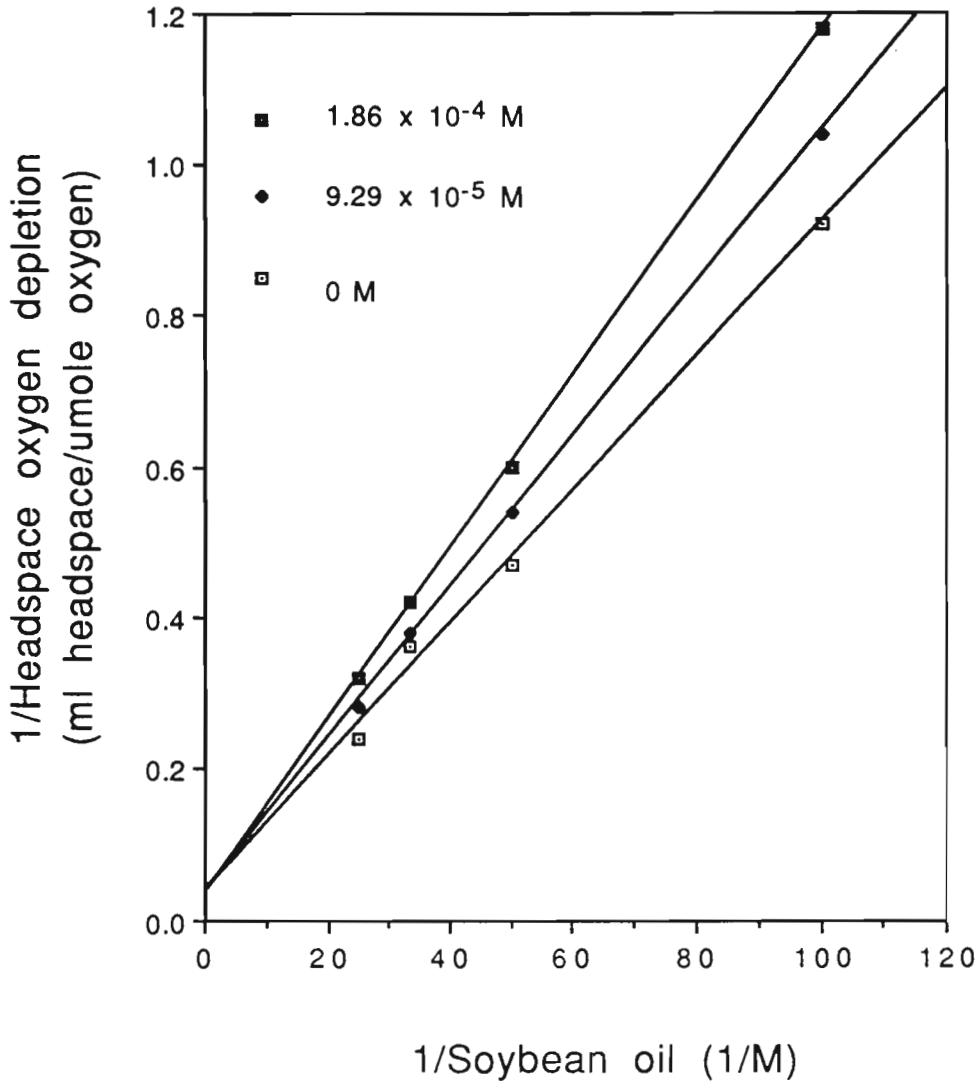


Fig. 16 - Effect of α-tocopherol on the headspace oxygen depletion of soybean oil containing 20 ppm Red No. 3 under light for 1 hr at 25°C

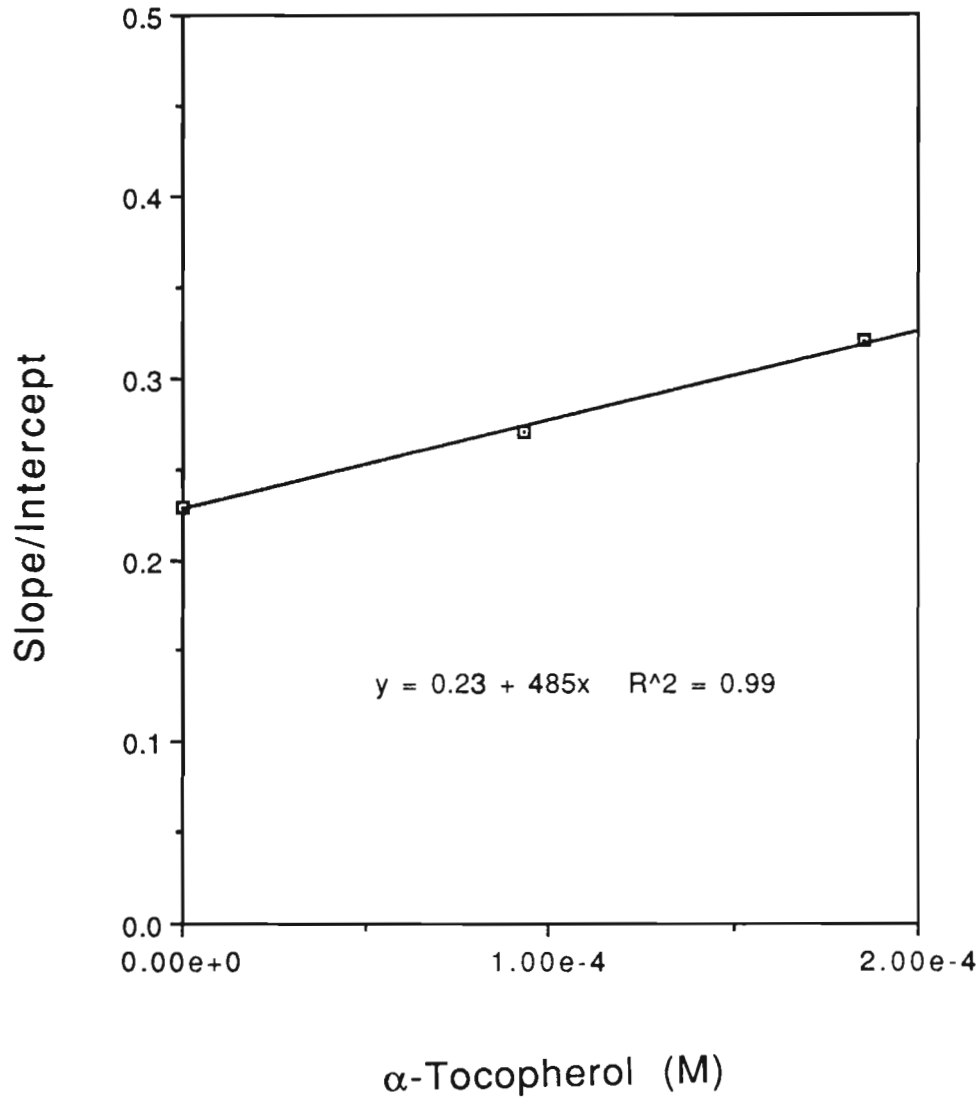


Fig. 17 - Regression line of slope/intercept of Fig. 16 vs. α-tocopherol concentrations

in chlorophyll photosensitized oxidation of soybean oil in methylene chloride (Jung et al., 1991), and $2.6x10^8$ $M^{-1}Sec^{-1}$ on methylene blue photosensitized oxidation of methyl linoleate in alcohol (Yamauchi and Matsushita, 1977). Foote (1976) reported that α-tocopherol quenched singlet oxygen at chemical quenching rate of $4.6x10^7$ $M^{-1}Sec^{-1}$ and physical quenching rate of $6.2x10^8$ $M^{-1}Sec^{-1}$ in methylene blue sensitized singlet oxygen formation in methanol. Since solvents affect the decay rate (Kd) of singlet oxygen (Hurst et al. 1983), the quenching rate may vary in different solvent systems.

6. Quenching Mechanisms and Kinetics of β-Carotene on the Red No. 3 Photosensitized Oxidation of Soybean Oil

The effects of 0, 0.93, 1.86, 3.72, and $7.44x10^{-6}$ M β-carotene on the headspace oxygen of 20 ppm Red No. 3 photosensitized oxidation of 0.01, 0.02, 0.03, and 0.04 M soybean oil are shown in Table 12. The headspace oxygen depletion of soybean oil in the absence of β-carotene is greater than that in the presence of β-carotene. As the concentration of β-carotene increased, the headspace oxygen depletion of soybean oil decreased. The headspace oxygen of the soybean oils containing Red No. 3 and β-carotene did not decrease under dark for 1 hr. The antioxidant effect of β-carotene on the Red No. 3 photosensitized soybean oil oxidation may be due to the quenching of singlet oxygen and/or the excited triplet Red No. 3 or both.

Table 12 - Effects of soybean oil and β-carotene concentrations on the headspace oxygen depletion of sample bottles containing Red No. 3 and soybean oil under light for 1 hr

Headspace oxygen depletion (μmole O_2/ml headspace)*					
	β-carotene (x10^{-6}M)				
Soybean oil	0	0.93	1.86	3.72	7.44
0.01M	1.10^{a}_{a}**	0.88^{a}_{b}	0.73^{a}_{c}	0.59^{a}_{d}	0.38^{a}_{e}
0.02M	2.20^{b}_{a}	1.75^{b}_{b}	1.34^{b}_{c}	1.11^{b}_{d}	0.60^{b}_{e}
0.03M	2.99^{c}_{a}	2.25^{c}_{b}	2.15^{c}_{b}	1.54^{c}_{c}	0.83^{c}_{d}
0.04M	3.84^{d}_{a}	3.15^{d}_{b}	2.77^{d}_{b}	1.92^{d}_{c}	1.06^{d}_{d}

* Mean of duplicate analyses.

* * The same superscript letters in the same column or the same subscript letters in the same row are not significantly different at $\alpha=0.05$.

The β-carotene quenching mechanisms and rates for singlet oxygen or triplet sensitizer were studied using steady-state kinetics. The kinetic equation of the formation rate of oxidized soybean oil in the presence of a Red No. 3 and β-carotene is

$$\{d[AO_2]/dt\}^{-1} = K^{-1}\{(Ko[O_2] + KQ[Q])/Ko[O_2]\}\{[Kr[A] + (Kox\text{-}Q + Kq)[Q] + Kd]/Kr[A]\}$$

The intercepts and slopes of $[AO_2]^{-1}$ vs. $[A]^{-1}$ at various [Q] of Fig. 18 are shown in Table 13. The intercepts of the regression lines of β-carotene at 0, $9.3x10^{-7}$ and $1.86x10^{-6}$ M were the same, but the slopes were different. The results indicated that β-carotene quenched singlet oxygen only. The equation, $\{d[AO_2]/dt\}^{-1} = K^{-1}\{Kd/Kr + (Kox\text{-}Q + Kq)[Q]/Kr\}$, can be applied to calculate the reaction rate of soybean oil with singlet oxygen and the β-carotene quenching rate for singlet oxygen. The plot of S/I vs. [β-carotene] gives an intercept, Kd/Kr and slope, (Kox-Q + Kq)/Kr (Fig. 19).

Table 13 - The intercepts and slopes of the plots of $[AO_2]^{-1}$ vs. $[A]^{-1}$ of Fig. 18 to determine the quenching mechanism and rate of β-carotene on the Red No. 3 photosensitized oxidation of soybean oil

β-carotene (M)	Intercept (ml headspace/μmole O_2)	Slope (M-ml headspace/μmole O_2)	S/I (M)
0	$3.48x10^{-2}$	$8.69x10^{-3}$	0.25
$9.3x10^{-7}$	$3.51x10^{-2}$	$1.10x10^{-2}$	0.32
$1.86x10^{-6}$	$3.20x10^{-2}$	$1.35x10^{-2}$	0.42
$3.72x10^{-6}$	0.132	$1.54x10^{-2}$	0.12
$7.44x10^{-6}$	0.459	$2.23x10^{-2}$	0.05

The intercept and slope were 0.25 M and $9.14.x10^{4}$, respectively. Kd is known as $1.96x10^{4}$ Sec^{-1} in acetone (Gorman and Rogers, 1989). Therefore, Kr is equal to $7.84.x10^{4}$ $M^{-1}Sec^{-1}$ and the total

singlet oxygen quenching rate constant (Kox-Q + Kq) is $7.3x10^9$ $M^{-1}Sec^{-1}$. The singlet oxygen quenching rate constants of carotenoids ranged from $5.72x10^9$ $M^{-1}Sec^{-1}$ to $9.79x10^9$ $M^{-1}Sec^{-1}$ in chlorophyll photosensitized oxidation of soybean oil using methylene chloride as a solvent (Lee and Min, 1990). As the β-carotene concentrations increased from 0 to $3.72x10^{-6}$ M and $7.44x10^{-6}$ M, the ratio of the slope to intercept of the plot decreased. The increase of intercept is greater than the increase of slope of the plot. The intercept represents the relative rate of singlet oxygen formation. The increase of the intercept as a function of the increased β-carotene suggests that β-carotene reduced the singlet oxygen formation by quenching the excited triplet Red No. 3. β-Carotene is frequently used as a diagnostic tool for the determination of singlet oxygen involvement in a chemical or biochemical oxidation reaction (Bellus, 1979). β-Carotene concentrations below the 10^{-3} M have been suggested not to quench excited triplet photosensitizers (Bellus, 1979). Min and Lee (1987) reported that β-carotene at the concentration of 9.3 $x10^{-6}$ to $3.71x10^{-5}$ M quenched singlet oxygen, but did not quench chlorophyll. The β-carotene at the concentration of $3.72x10^{-6}$ M and 7.44 $x10^{-6}$ M quenched Red No. 3 in this study. The triplet Red No. 3 quenching by β-carotene has not been reported. β-Carotene effectively quenched singlet oxygen and triplet Red No. 3 at the concentration of $3.72x10^{-6}$ M and $7.44x10^{-6}$ M in this study. The quenching rate of β-carotene on the excited triplet Red No. 3 molecules can not be obtained from the equation, $\{d[AO2]/dt\}^{-1} = K$-

^{1}\{(Ko[O2] + KQ[Q])/Ko[O2]\}\{[Kr[A] + (Kox-Q + Kq)[Q] + Kd]/Kr[A]\}, because Ko which is the reaction rate constant of excited triplet Red No. 3 with triplet oxygen is unknown. The excited triplet Red No. 3 quenching rate by β-carotene can be indirectly determined by measuring the singlet oxygen formation. The singlet oxygen is formed by the excited triplet sensitizer-triplet oxygen annihilation. The effects of β-carotene on the formation of singlet oxygen in the presence of 20 ppm Red No. 3 under light for 1 hr are shown in Table 14. As the β-carotene concentration increased from 0 to 9.30×10^{-7}, 1.86×10^{-6}, 3.72×10^{-6}, and 7.44×10^{-6}M, the relative singlet oxygen yield decreased from 100 to 8 %

Table 14 - The effects of β-carotene on the formation of singlet oxygen in the presence of 20 ppm Red No. 3 under light for 1 hr

β-Carotene (M)	Relative singlet oxygen formation (%)
0	100
9.30×10^{-7}	100
1.86×10^{-6}	100
3.72×10^{-6}	26
7.44×10^{-6}	8

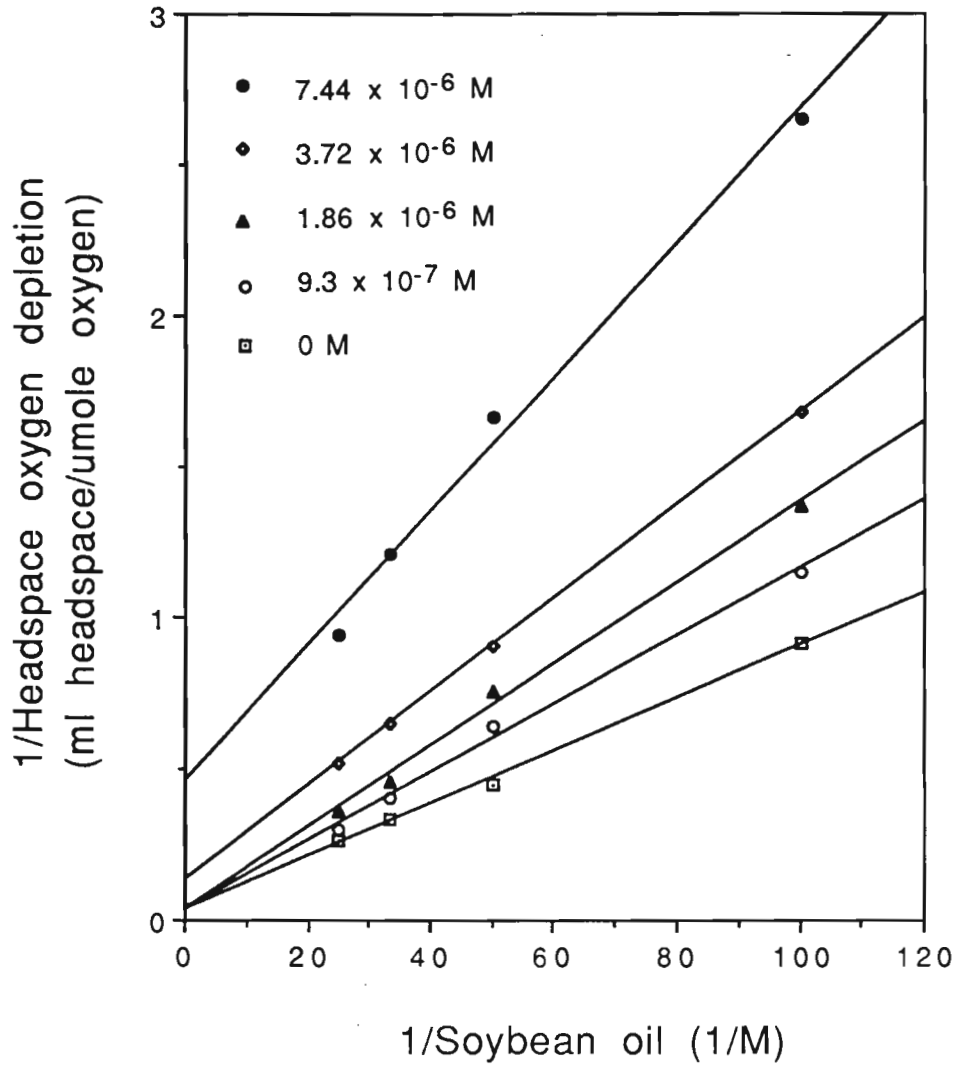


Fig. 18 - Effect of β-carotene on the headspace oxygen depletion of soybean oil containing 20 ppm Red No. 3 under light for 1 hr at 25°C

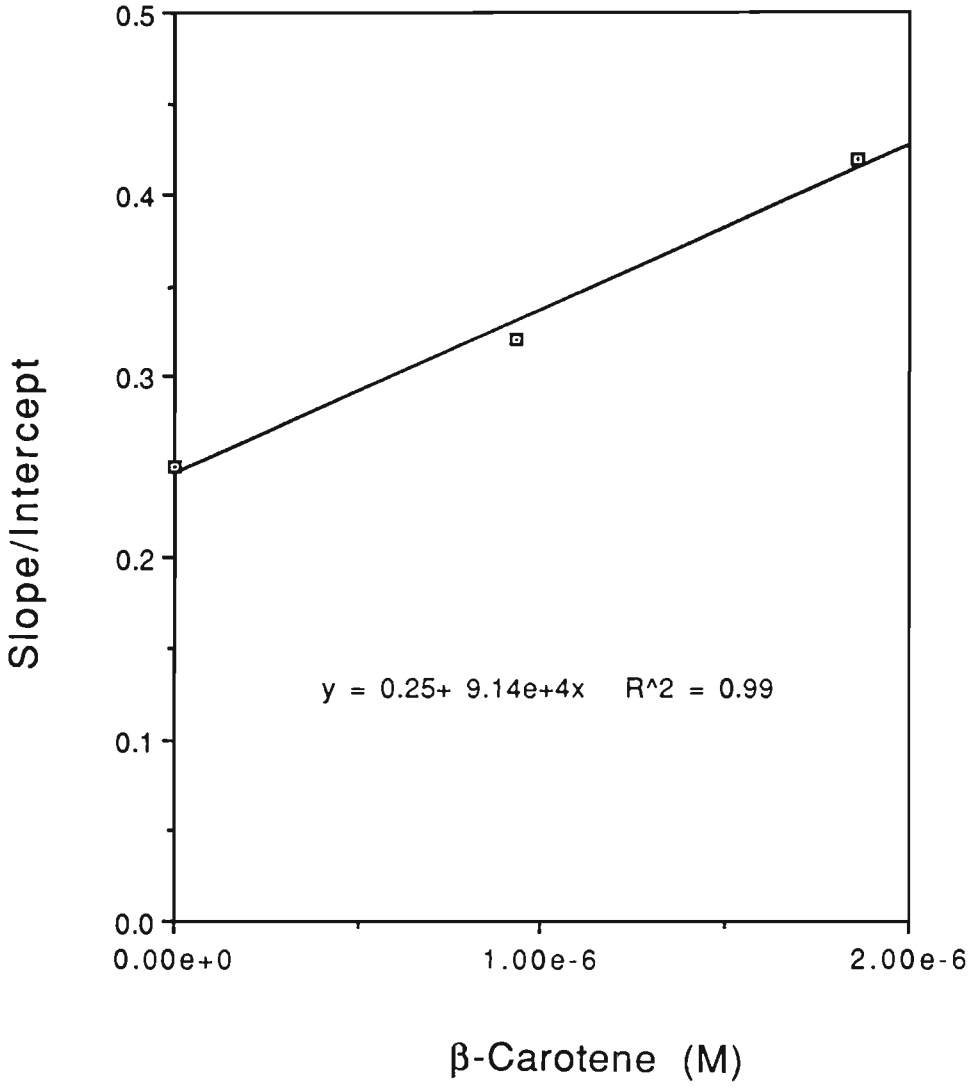


Fig. 19 - Regression line of slope/intercept of Flg. 18 vs. β-carotene concentrations

7. Determination of Ascorbic Acid Contents by Spectrophotometry

The absorbances of standard 20, 40, 60, 80, or 100 ppm ascorbic acid solutions at 265 nm were determined by spectrophotometry. Fig. 20 shows a linear relationship between the absorbances and ascorbic acid concentrations. The correlation coefficient (R^2) was 0.99.

8. Effects of pH on the Oxidation of Red No. 3 and Ascorbic Acid in the Presence of Red No. 3 under Dark and Light

The pH has been reported to affect the oxidation of ascorbic acid (Hughes, 1985). The pH from 3.52 to 7.22 is commonly used in vitamin formulations and other applications of ascorbic acid in food systems (Blaug and Hajratwala, 1972). The samples containing 0, 50, 100, 150, and 200 ppm ascorbic acid at pH 4, 5.6, or 7 with and without 40 ppm Red No. 3 were stored under light and dark for 1 hr. The pH effects on the 40 ppm Red No. 3 before and after the storage under dark and light for 1 hr are shown in Table 15. The concentrations of Red No. 3 solution did not change at pH 4, 5.6, and 7 under dark for 1 hr, which indicated pH had no effect on Red No. 3 solution under dark. The concentration of 40 ppm Red No. 3 at pH 4 decreased from 40 to 22 ppm, but the concentrations of 40 ppm Red No. 3 at pH 5.6 or pH 7 were essentially the same under light for 1 hr. The result indicated

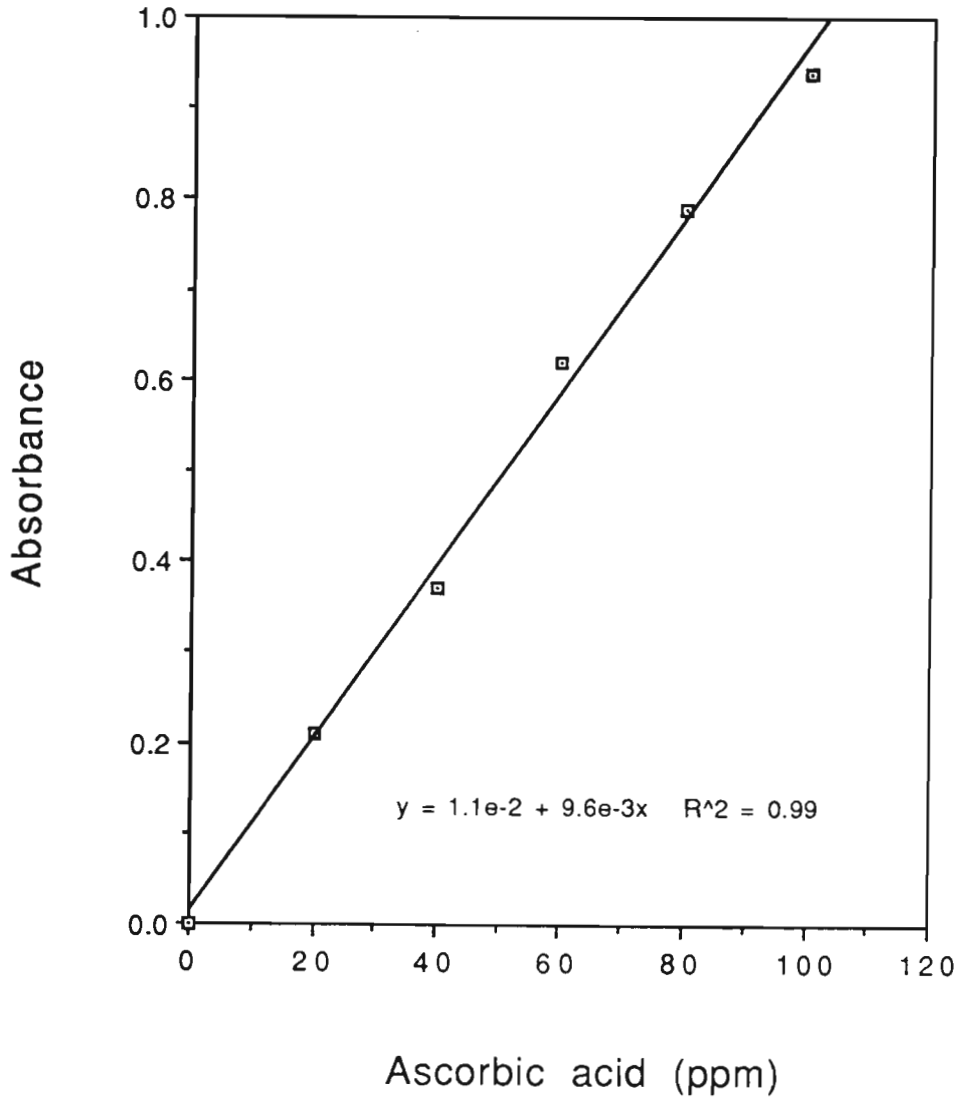


Fig. 20 - Correlation of absorbances at 265 nm and ascorbic acid concentrations

that Red No. 3 was stable at pH 7 and 5.6, but was not stable at pH 4 under light.

Table 15 - The effect of pH on the 40 ppm Red No. 3 solution under dark and light for 1 hr

	Concentration (ppm)*		
pH	Before storage	Under dark	Under light
4.0	40	40	22
5.6	40	40	37
7.0	40	40	40

* Mean of triplicate analyses

Satter et al. (1977) reported that fluorescence light had no effect on the ascorbic acid loss at the concentrations of 300-1500 ppm under light. The light effect on the loss of ascorbic acid occurred in the presence of metal ions or riboflavin. The effects of 40 ppm Red No. 3 on the 50, 100, 150, and 200 ppm ascorbic acid at pH 4, 5.6, and 7 under light storage for 1 hr are shown in Table 16. The absorbance due to 40 ppm Red No. 3 at 265 nm was about 8% of the absorbance of sample. The absorbance due to 40 ppm Red No. 3 was subtracted from the absorbance of the sample to determine the absorbance and contents of ascorbic acid of sample. The loss of ascorbic acid in the absence of Red No. 3 increased as the pH increased from 4 to 5.6 and 7. The relative rate of ascorbic acid loss increased as ascorbic acid

concentration increased. The pH greatly affects the presence of dominant ions of ascorbic acid in aqueous solution which may affect the oxidation rate. The degradation of dehydroascorbic acid to 2,3-diketogulonic acid is also pH dependent (Hughes, 1985). Blaug and Hajratwala (1972) reported that phosphate ions had catalytic activity in the oxidation of ascorbic acid in the range of pH 5.85 - 7.22. The relative content of phosphate of citrate-phosphate buffer increased in higher pH and the increase might cause the loss of ascorbic acid at higher pH in the absence of Red No. 3 during light storage. The Red No. 3 accelerated the oxidation of ascorbic acid at pH 4, 5.6 and 7 (Table 16). The loss of 50, 100, 150, 200 ppm ascorbic acid at pH 4 in the presence of 40 ppm Red No. 3 were 98, 91, 75, and 63 %, respectively. Similarly, the loss of ascorbic acid of 50, 100, 150, 200 ppm ascorbic acid at pH 5.6 were 99, 94, 83, and 69 %, respectively, and 100, 95, 82, 70%, respectively in the presence of 40 ppm Red No. 3 at pH 7. The results indicated that 50 and 100 ppm ascorbic acid at pH 4, 5.6, and 7 during light storage were almost destroyed in the presence of 40 ppm Red No. 3 (Table 16). The pH effect on the 50 ppm ascorbic acid solution in the presence of Red No. 3 under dark for 1 hr is shown in Table 17. The ascorbic acid content at pH 4.0, 5.6, and 7.0 in the presence of 40 ppm Red No. 3 under dark did not change. The pH and Red No. 3 did not cause the loss of ascorbic acid under dark for 1 hr. The effect of Red No. 3 on ascorbic acid was due to singlet oxygen oxidation of ascorbic acid under light (Table 16). The loss of ascorbic acid at pH 4 due to singlet oxygen

Table 16 - The effects of 40 ppm Red No. 3 on the ascorbic acid solution at pH 4.0, 5.6 and 7.0 under light for 1 hr

	Loss of ascorbic acid (ppm)		
Ascorbic acid (ppm)	Red No. 3 (0 ppm)	Red No. 3 (40 ppm)	Net loss (due to Red No. 3)
pH 4.0			
50	7.8	48.8	41.0
100	11.1	91.3	80.2
150	15.8	112.2	97.4
200	22.3	126.1	103.7
pH 5.6			
50	8.3	49.6	41.3
100	15.5	94.2	78.7
150	31.2	124.0	93.2
200	37.2	138.3	101.1
pH 7.0			
50	18.2	50.0	31.8
100	34.3	95.1	60.8
150	45.8	122.3	76.5
200	50.4	141.0	90.6

* Mean of triplicate analyses

oxidation was about 5 times greater than that of autoxidation under light for 1 hr. Red No. 3 acted as a photosensitizer to accelerate the oxidation of soybean oil under light in our previous soybean oil study.

Table 17 - The effect of pH on the 50 ppm ascorbic acid solution in the presence of 40 ppm Red No. 3 under dark for 1 hr

	Concentration (ppm)*	
pH	Before storage	after storage
4.0	50.0	50.0
5.6	50.0	49.8
7.0	50.0	49.4

* Mean of triplicate analyses

9. Determination of the Reaction Rate Constants of Singlet Oxygen Oxidation of Ascorbic Acid

The loss of ascorbic acid by the Red No. 3 photosensitized singlet oxygen oxidation was measured by spectrophotometry at 265 nm. The reaction rate constant of singlet oxygen with ascorbic acid at pH 4, 5.6, and 7 can be calculated by measuring the spectrometric absorbance of ascorbic acid at 265 nm and using the kinetic equation, $\{d[AO_2]/dt\}^{-1} = K^{-1}\{1 + [(Kox\text{-}Q + Kq)[Q] + Kd]/Kr[A]\}$. Since there is no singlet oxygen quencher used, the equation can be further simplified to the following:

$\{d[AO2]/dt\}^{-1} = K^{-1}\{1 + Kd/Kr[A]\}$

Where $d[AO_2]/dt$ is the reaction rate of singlet oxygen with ascorbic acid; K^{-1}, formation rate of singlet oxygen; Kd, decay rate constant of singlet oxygen in water; Kr, reaction rate constant of ascorbic acid with singlet oxygen. The plot of the reciprocal of reaction rate vs. the reciprocal of concentrations of ascorbic acid gives a slope/intercept, kd/kr. Kd is $2.5x10^5$ Sec^{-1} in water (Gorman and Rogers, 1989); therefore, Kr can be calculated. The plots of the reciprocal of reaction rate vs. the reciprocal ascorbic acid at pH 4, 5.6 and 7 are shown Figs. 21, 22, and 23, respectively. The intercepts and slopes of plots are listed in Table 18. The slope/intercept of the plot is kd/kr. Kd is $2.5x10^5$ Sec^{-1} in water. Kr values for the reaction rate constant of ascorbic acid with singlet oxygen were $1.53x10^8$, $1.86x10^8$, and $1.19x10^8$ $M^{-1}Sec^{-1}$ at pH 4, 5.6, and 7, respectively.

Table 18 - The intercepts and slopes of the plots at pH 4.0, 5.6, and 7.0 of Figs. 21, 22, and 23 to determine the reaction rate constants of singlet oxygen oxidation of ascorbic acid

pH	Intercept (Sec/M)	Slope (Sec)	Slope/Intercept (M)
4.0	$2.26x10^6$	$3.68x10^3$	$1.63x10^{-3}$
5.6	$2.63x10^6$	$3.53x10^3$	1.34×10^{-3}
7.0	$2.36x10^6$	$4.94x10^3$	$2.09x10^{-3}$

The reaction rate constants of singlet oxygen with ascorbic acid at pH 4, 5.6, and 7.0 were different, which indicated that pH from 4 to 7 had an effect on the singlet oxygen oxidation of ascorbic acid in the presence of 40 ppm Red No. 3 under light.

10. Determination of the Reaction Rate Constants of Singlet Oxygen Oxidation of Ascorbic Acid by Headspace Oxygen Analysis

The effects of 40 ppm Red No. 3 on the headspace oxygen of 50, 100, 150, and 200 ppm ascorbic acid at pH 7 under light for 1 hr are shown in Table 19.

Table 19 - The effect of 40 ppm Red No. 3 on the headspace oxygen depletion of ascorbic acid samples under light for 1 hr

	Depletion of headspace oxygen (μmole O2/ml headspace)*		
Ascorbic acid (ppm)	Red No. 3 (0 ppm)	Red No. 3 (40 ppm)	Net loss (due to Red No. 3)
50 (ppm)	0.10^{a**}	0.49^{a}	0.39^{a}
100	0.18^{b}	0.84^{b}	0.65^{b}
150	0.27^{c}	1.03^{c}	0.76^{c}
200	0.29^{c}	1.15^{d}	0.86^{d}

* Mean of duplicate analyses.

* * Mean values in the same column with different superscript letters are significant at α=0.05.

The results indicated that as the ascorbic acid concentration increased, the headspace oxygen depletion increased. The headspace oxygen of the sample containing 40 ppm Red No. 3 did not change under dark for 1 hr. The loss of ascorbic acid in the presence of Red No. 3 under light for 1 hr was approximately 3.4 fold greater than that in the absence of Red No. 3. The effect of Red No. 3 on the loss of ascorbic acid under light was due to singlet oxygen oxidation of ascorbic acid. The reaction rate constant of singlet oxygen with ascorbic acid can be calculated by using the following kinetic equation:

$$\{d[AO_2]/dt\}^{-1} = K^{-1}\{1 + Kd/Kr[A]\}$$

Where $d[AO_2]/dt$ is the reaction rate of singlet oxygen with ascorbic acid. The plot of the reciprocal of reaction rate vs. the reciprocal of various concentrations of ascorbic acid gives an y-intercept and slope of the regression line (Fig. 24). The y-intercept was the reciprocal of singlet oxygen formation rate in ascorbic acid solution containing 40 ppm Red No. 3; therefore, the singlet oxygen formation rate was 1.5 μmole oxygen/ml headspace/hr. The slope/intercept, kd/kr, of the plot in Fig. 24 was 8.1×10^{-4} M. Kd is 2.5×10^{5} Sec^{-1} in water (Gorman and Rogers, 1989), so Kr was 3.08×10^{8} $M^{-1}Sec^{-1}$. Kr from the headspace oxygen method is higher than that from the direct measurement of ascorbic acid loss. The reason might be partly due to the further oxidation of the oxidized ascorbic acid (Hughes, 1985). The Kr is

the chemical quenching rate of singlet oxygen by ascorbic acid. The Kr for soybean oil, α-tocopherol and β-carotene were $8.2x10^4$, $4.1x10^7$, $7.3x10^9$ $M^{-1}Sec^{-1}$, respectively. The singlet oxygen quenching rate of ascorbic acid indicated that ascorbic acid is a very good singlet oxygen quencher and can be used in food systems to minimize singlet oxygen oxidation.

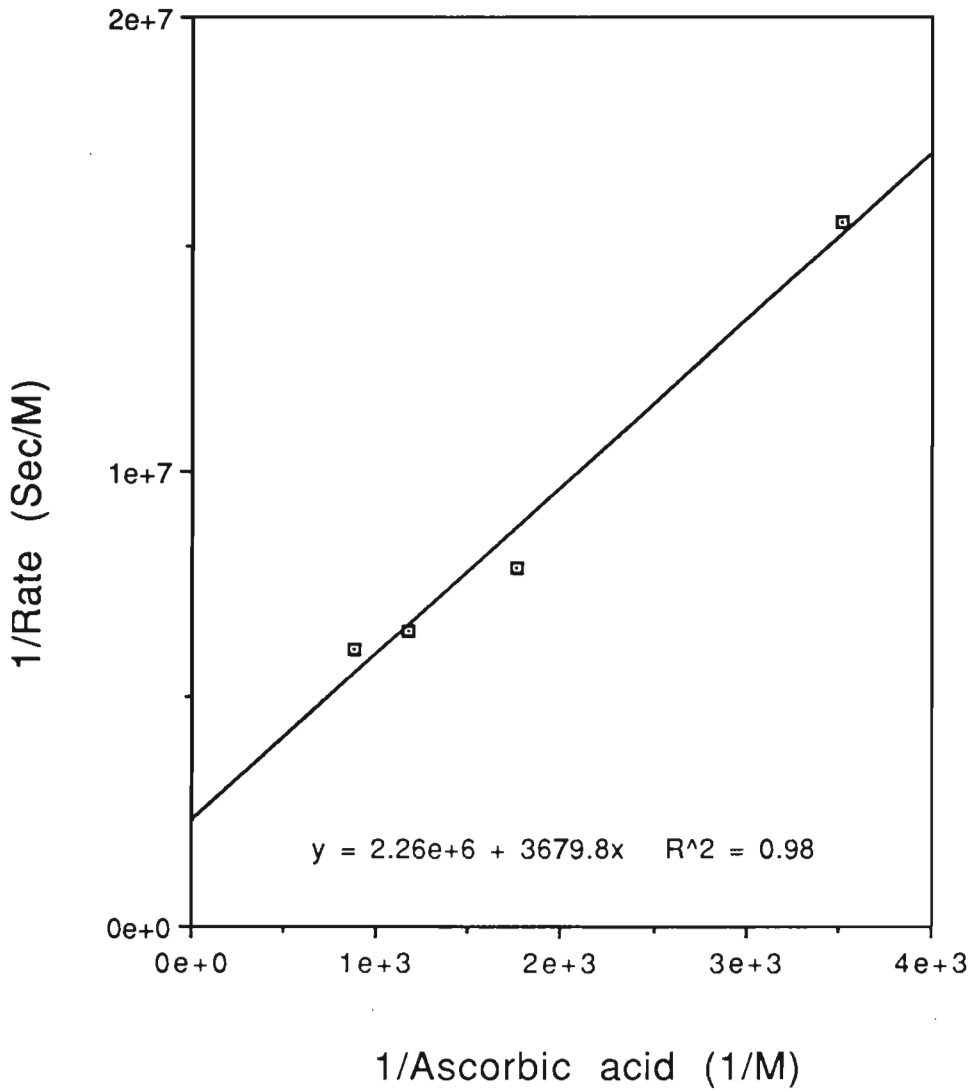


Fig. 21 - Rate of ascorbic acid loss vs. ascorbic acid concentrations at pH 4.0 in the presence of 40 ppm Red No. 3 under light for 1 hr

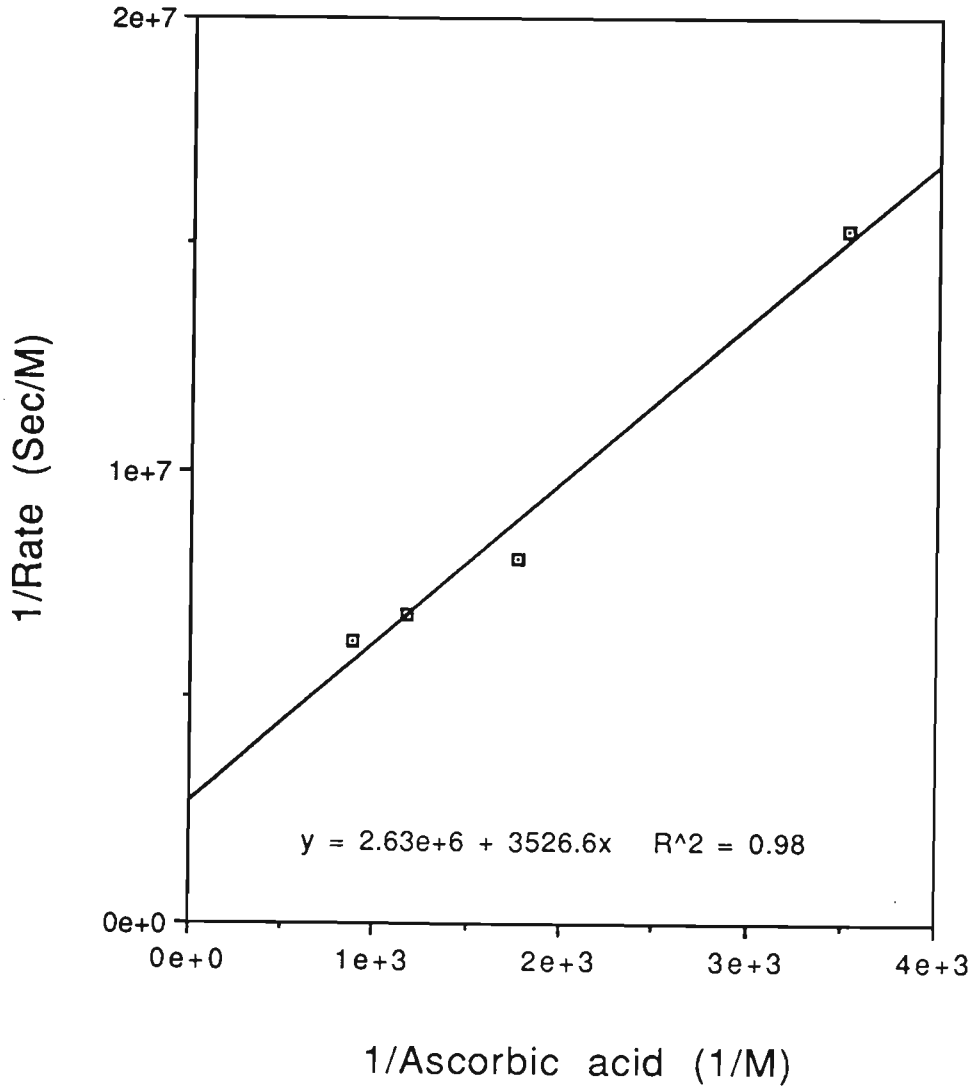


Fig. 22 - Rate of ascorbic acid loss vs. ascorbic acid concentrations at pH 5.6 in the presence of 40 ppm Red No. 3 under light for 1 hr

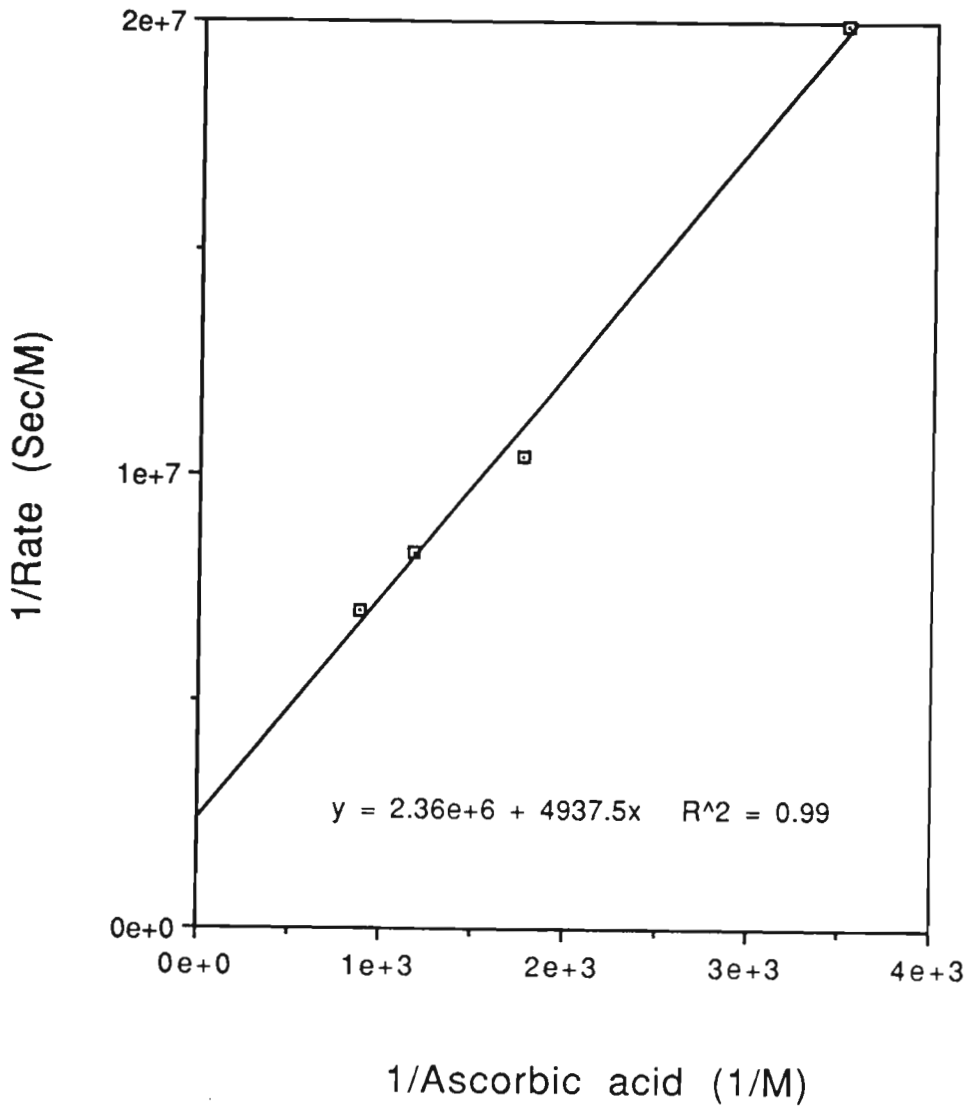


Fig. 23 - Rate of ascorbic acid loss vs. ascorbic acid concentrations at pH 7.0 in the presence of 40 ppm Red No. 3 under light for 1 hr

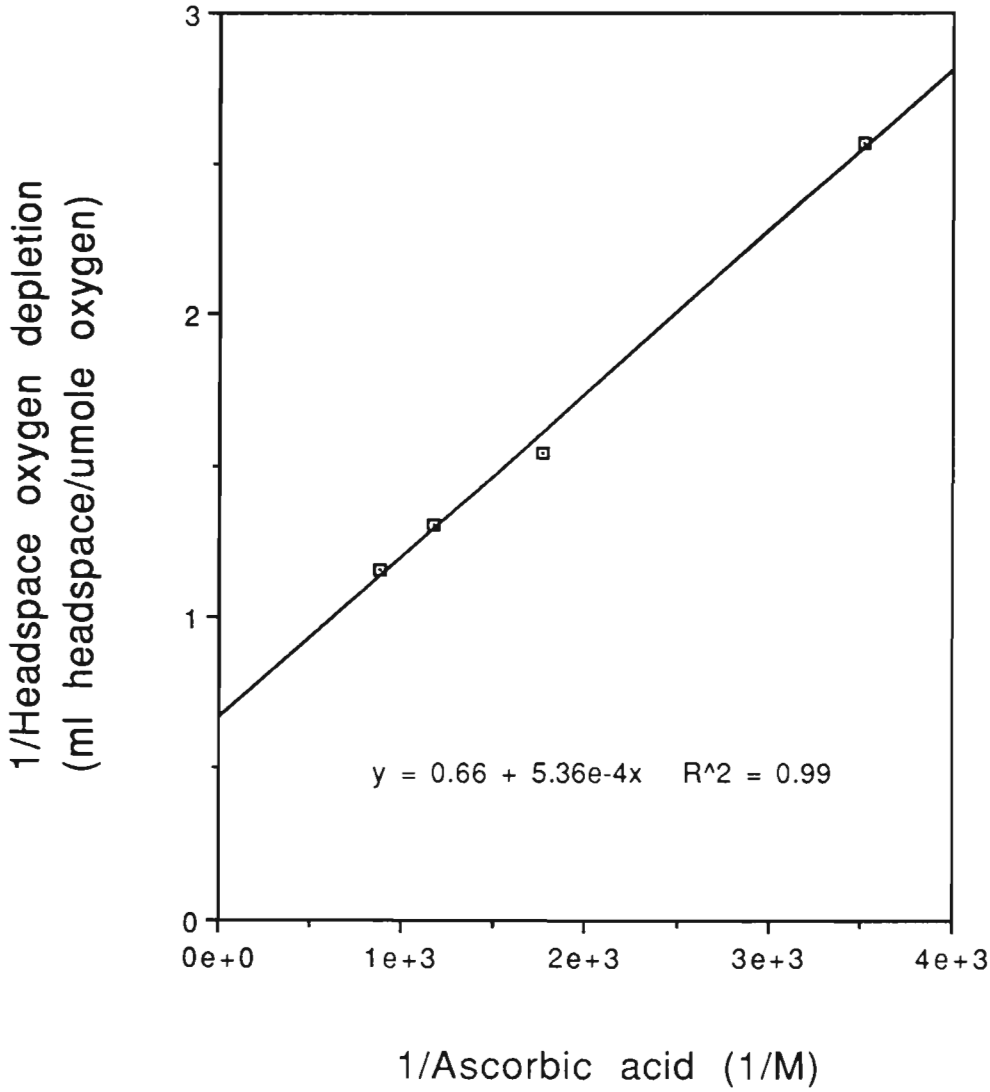


Fig. 24 - Reciprocal of headspace oxygen depletion vs. ascorbic acid concentrations in the presence of 40 ppm Red No. 3 under light for 1 hr

V. CONCLUSIONS

(1) A light box which was lined with mirrors on the inside walls and equipped with a motor-driven sample holder was designed. It provided uniform light to the samples during storage and improved reproducibility of sample results. The coefficient variation of the headspace analyses of samples was reduced from 8 % in the preliminary study with the old light box to 0.55% with the new light box.

(2) A stable oil/water/acetone solution was developed to study the effects of water soluble colorants, α-tocopherol and β-carotene on the photosensitized lipid oxidation.

(3) The effects of Red No. 3, Eosin B, rose bengal, methlyene blue of which the absorption spectra range from 435 to 750 nm on soybean oil indicated no special visible spectrum required for a colorant to be a photosensitizer. The prooxidant activity of the colorants on the oxidation of soybean oil under light was in the order of methlyene blue > rose bengal > Red No. 3 > Eosin B.

(4) Red No. 3 was the only photosensitizer among FD&C Red No. 40, Red No. 3, Yellow No. 5, Yellow No. 6, Green No. 3, Blue No. 1, and Blue No. 2 to initiate singlet oxygen oxidation of soybean oil.

(5) α-Tocopherol and β-carotene were effective antioxidants in Red No. 3 photosensitized oxidation of soybean oil. The singlet oxygen quenching rates of α-tocopherol and β-carotene were 4.1×10^7 and 7.3×10^9 $M^{-1}Sec^{-1}$, respectively.

(6) The kinetic study of quenching mechanisms showed α-tocopherol quenched singlet oxygen rather than triplet Red No. 3. β-Carotene quenched singlet oxygen only in the Red No. 3 photosensitized lipid oxidation at or below the $1.86X10^{-6}M$ and quenched triplet Red No. 3. at or above the concentration of $3.72\ X10^{-6}M$.

(7) The pH affected the autoxidation of ascorbic acid under light for 1 hr , but had no effect under dark. The oxidative stability of ascorbic acid decreased as the pH increased from 4, to 5.6 and 7 under light.

(8) Red No. 3 accelerated the oxidation of ascorbic acid under light, but did not have any effect under dark. The loss of ascorbic acid at pH 4 due to singlet oxygen oxidation was

about 5 times greater than the autoxidation under light for 1 hr.

(9) The kinetic study of pH 4.0, 5.6, and 7.0 on the ascorbic acid oxidation in the presence of 40 ppm Red No. 3 under light showed that pH had an effect on singlet oxygen oxidation of ascorbic acid.

(10) The singlet oxygen formation rate of the 40 ppm Red No. 3 photosensitized oxidation of ascorbic acid in pH 7 solution was 1.5 μmole oxygen/ml headspace/hr.

(11) The average reaction rate of ascorbic acid with singlet oxygen in pH 4.0, 5.6 and 7.0 solutions was 1.4×10^8 $M^{-1}Sec^{-1}$. Therefore, ascorbic acid is an effective singlet oxygen quencher.

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