Thesis for Candidate (Ph.D) Degree

Antioxidant Function of Tocopherol, Ascorbic Acid and Carotenoids in Food and Model Systems.

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CONTENTS

1. INTRODUCTION

The shelf-life of foods is often limited. Their stability is, in general, restricted due to reactions such as oxidative degradation of lipids, browning (Maillard reaction) and retrogradation of starch. Due to these processes, loss of structure, color and flavor is often observed during maturation, processing and storage of foodstuffs.

In connection with a variety of pathological events and diseases such as cancer and heart diseases, lipid oxidation has aroused great attention lately. It has become evident that oxidized lipids promotes cancer onset in human body (Gey et al. 1989, Simon et al. 1992, Gerster et al. 1991 and Garewal et al. 1995). The role antioxidant vitamins play in the prevention of cancer has been researched throughout the world for well over a decade (Albanes et al. 1995 and Hennekens et al. 1995). It has been postulated that the oxidation damage to DNA, proteins and other macromolecules causes the degenerative disease which associated with aging, cancer, cardiovascular disease, immune-system decline, brain dysfunction and cataracts (Ames et al. 1993). Originating with the observations of lower risk of cancer in persons consuming more fruits and vegetables, prevention of cancer in human body has been demonstrated to be dependent on the antioxidation potency in the different organs (Ziegler et al. 1989, Steinmetz et al. 1991 and Block, 1992). Most recently, carotenoids have been found cancer preventive metabolites, therefore, current research work is being focused on them all over the world.

Over 100 observational epidemiological studies have assessed the relationship of dietary antioxidant intake or blood nutrient levels with cancer

risk. Such studies are not entirely consistent but provide support for the hypothesis that antioxidant vitamin intake may decrease cancer risk. However, the chief limitation of such observational studies is their inability to control for all factors associated with vitamin intake that might independently affect cancer risk. Such unknown or unmeasured confounding variables could account for all or part of any observed associations. For this reason, definitive data on the role of antioxidant vitamins and cancer can derive only from properly conducted large-scale randomized trials of sufficient sample size, dose and duration of treatment and follow-up. Over the next several years, these trials should provide clear evidence concerning the role of antioxidant vitamins in the prevention of cancer. Such data are crucial for individual clinical decision making as well as rational public health policy (Hennekens et al. 1994).

Data available on this topic implied that effectiveness of every one of these antioxidants depends upon their ability to interfere with the free radical cycle through which oxidation process widespread in the whole biological system (Bruckdorfer et al. 1990 and Sies, 1991).

Although, lipid oxidation is a chemical reaction with a low activation energy, the rate of this reaction is not significantly diminished by lowering the temperature of the storage (Hamilton et al. 1983 and Labuza et al. 1971). To overcome this problem, synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hidroxytoluene (BHT) are often incorporated in the edible oils. However, there is a decline in their usage (Dziezak, 1986) probably because of the consumers' preference for natural

antioxidants. In the last 15-20 years, special attention has been given to the use of natural antioxidants because of the possible, but not well established, hazardous effects of synthetic antioxidants, and because of the worldwide trend to avoid or minimize the use of food additives (Frenkel, 1993).

The level of the essential antioxidant vitamins is, in contrast to other antioxidative defense, mainly determined by their dietary supply. Fruits and vegetables are the main sources of antioxidant vitamins that make these foods essential to human health. Furthermore some environmental and variety factors can initiate unfavorable changes in the chemical composition of the fruits and vegetables that makes the analytical survey, from time to time, of great significance (Han et al. 1990).

The contamination of urban areas is dramatically increased from day to day as a result of traffic and industrial pollution. Of hazardous pollutants heavy and toxic metals are of special interest in Europe. The level of such metals is increasing in the air, water and soil of the agricultural areas. The high uptake by plants of such metals may lead to death of plants at a certain growth stage or initiate undesirable changes in the composition of the food that make it completely unacceptable from the nutritional and food safety points of view.

Lipid oxidation leading to rancidity is often the decisive factor determining the useful storage life of food products, even when their fat content is very low. Much research has been conducted to understand better the mechanism of oxidation of polyunsaturated lipids, antioxidant action, and

the effects of decomposition products of lipid oxidation on the development of rancidity in foods. Recent evidence indicates, however, that animal cancer tests involve much guesswork and cannot be used to predict absolute human risks. Because such studies are costly many firms have chosen to eliminate the use of antioxidant additives to save on testing costs (Frenkel et al. 1989).

In different food systems effective natural antioxidants exhibit different antioxidation potencies and sometimes, depending on the conditions of food systems, they are absolutely ineffective. Factors most likely to affect efficiency of bioantioxidants may include water activity, presence or absence of other antioxidants and oxygen pressure in the system.

Among food constituents fat-soluble pigments (carotenoids and chlorophylls) extremely interfere with lipid oxidation. These pigments are cooxidized to colorless compounds via free radical chain reactions. By such a mechanism some of them can perform antioxidation function, thereby interacting with other antioxidants. It is, therefore of interest to investigate the interaction between such metabolites in different systems by model experiments.

The objectives of this work are:

1. Analytical highlight on fat-soluble pigments and natural antioxidants in some fruits and vegetables.
2. Studying the interaction between fat-soluble pigments and natural antioxidants during ripeness and storage of food.

3. Investigating the effect of environmental pollution with toxic and heavy metals on the pigment and antioxidant content of spinach leaves.
4. Elaboration of new model systems for investigating the antioxidation potency of some effective natural antioxidants through accelerated oxidation of linoleic acid in aqueous and non-aqueous systems.
5. Study of oxidative degradation of carotenoids from different sources in the presence and absence of effective natural antioxidants.
6. Estimation of effective concentration of natural antioxidants under different conditions using cooxidation of pigment as a bioindicator.

2. LITERATURE REVIEW

2.1 Lipid peroxidation in foods

The problem of ensuring a high quality of lipids and lipid-containing products and prolonging their storage time is directly associated with their optimum stabilization by the addition of suitable antioxidants, gassing with inert gases, appropriate packaging, special formulation, etc. (Marinova et al. 1992 and Loliger et al. 1993). It has become evident that consumption of food containing considerable amount of oxidized lipids can impair human health. Recently, in connection with a variety of diseases and pathological events (e.g. aging, asthma, cancer, etc.) lipid oxidation has received much attention (Basaga et al. 1990 and Poli et al. 1989).

Lipid peroxidation (or autoxidation) is a chain reaction that proceeds in three stages: initiation, propagation and termination stages. In initiation phase (Reaction 1), carbon-centered lipid radicals, $R^{\bullet}$, are produced by the abstraction from, or addition to, a polyunsaturated fatty acid, RH, of an oxy radical generated elsewhere (e.g. by the decomposition of a hydroperoxide, ROOH).

Initiation: production of $R^{\bullet}$ (carbon-centered radical). (1).

There are two groups of reactions involved in initiating autoxidation. The first group is confined to the initiating reactions which overcome the energy barrier required for the reaction of molecular oxygen with an unsaturated fatty acid. This group of reactions includes photosensitized oxidation (photooxidation) and lipid oxidation by lipoxygenase catalysis.

Both reactions provide the „first" primary hydroperoxides. These are then converted further into radicals by the second group of reactions. Heavy metal ions and heme (in) proteins are involved in this second reaction group. Some enzymes which generate the radical anion can be placed in between these two delineated reaction groups since at least H_2O_2 is in addition necessary as reactant for the formation of radicals (Belitz et al. 1992).

In the propagation phase (Reaction 2 and 3), the carbon-centered radical reacts rapidly with molecular oxygen to form a peroxyl radical ($ROO^{\bullet}$), a chain-carrying radical that able to attack another polyunsaturated lipid molecule. Although the initial peroxyl radical is converted to a hydroperoxide (ROOH), this process produces a new carbon centered radical, which is rapidly converted by Reaction 2 into another peroxyl radical.

Propagation: $R^{\bullet} + O2 \longrightarrow ROO^{\bullet}$ **(fast). (2)**

$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$ **(H atom abstraction).(3).**

The propagative process continues and can become a runaway process, consuming valuable polyunsaturated fat and producing a corresponding quantity of hydroperoxide (ROOH). The chain reaction does not stop until the chain-carrying peroxyl ($ROO^{\bullet}$) meets and combines with another peroxyl radical to form inactive products (reaction 4), termination reactions 5 and 6

play a role when, for example, the oxygen level is low, e.g. in the inner portion of a food.

Termination : $ROO^{\bullet} + ROO^{\bullet} \longrightarrow$ **inactive products. (4)**

$R^{\bullet} + ROO^{\bullet} \longrightarrow$ **inactive products. (5)**

$R^{\bullet} + R^{\bullet} \longrightarrow$ **inactive products. (6)**

The hypothesis presented in the reactions 1-6 is valid only for the initiation phase of autoxidation. The process becomes less and less clear with increasing reaction time since, in addition to hydroperoxides, secondary products are relatively unstable. They enter into numerous and complex breakdown and interaction mechanisms responsible for the production of myriad compounds of various molecular weights, flavor thresholds, and biological significance (Farmer et al. 1942. Burton et al. 1989 and Yamamoto et al. 1985).

2.2 Cooxidation of pigments

Pigments in foods are subjected to rapid decomposition in the presence of some catalysts such as oxygen, metals, enzymes and light. This decomposition often leads to concurrent loss of color and biological activity of the pigments. Presence of hydroperoxides and carbonyl compounds among products of oxidative decomposition of fat soluble pigments reveal their interference with the free radical cycles of lipid peroxidation.

It was earlier believed that oxidative decomposition of chlorophyll is light-dependent process. Progress in the bioanalytical methods opened up the possibilities for new findings in this field have become repeatedly evident that all types of oxidative degradation are chain radical reactions in which initiators and inhibitors are involved (Goldman et al. 1983).

The carotenoids are easily cooxidizable when exposed to a free radical chain reaction (Biacs et al. 1993) and a small amount of radical initially formed in vivo, either by enzyme-catalyzed or non-enzymatic oxidation, may cause alteration to their chemical and biological properties as shown in Figure 1. The lipoxygenase can cooxidize carotenoids and chlorophylls and thus can degrade these pigments to colorless products. The involvement of the enzyme in cooxidation reactions can be explained by the possibility that the peroxy radicals are not fully converted to their hydroperoxides. Thus, a fraction of the free peroxy radicals is released by the enzyme. It can abstract an H-atom from unsaturated fatty acid present or from a polyene (Klein et al. 1985).

Different methods were used by the research workers to evaluate the degradation of carotenoids. Hung-En et al. (1972), Teixeira et al. (1981), Haralampu and Karel (1983), Goldman et al. (1983) and Minguez et al. (1995) used spectrophotometer procedure to determine carotenoids degradation, while HPLC was used by others (Zakaria et al. 1979, Hsieh et al. 1983, Pesek et al. 1987 and Biacs et al. 1993).

2.2.1 Photooxidative degradation

In different food systems the loss of fat-soluble pigments as a function of exposure to light (photooxidative degradation) has been found to be associated with the unsaturation and inclusion of degree of lipid singlet oxygen into the process (Carnevale et al. 1979 and Teixeiraneto et al. 1981).

As concern carotenoids, it has been proposed that these components may enter into different reactions of oxidative degradation functioning either as quenching agents and inhibitor of spontaneous fat oxidation or as substrates for degradation. As for chlorophylls many researches emphasized their role as sensitizer for photoisomerization reactions of carotenoids (Lee and Min, 1988, O'Neil and Schwartz, 1995).

The liability of the carotenoid molecules is due to its conditions as a chromaphores when absorb light energy. Provision of energy can break the conjugation sequences, possibly by promoting the electrons to anti-bonding orbital, and this brings about the loss of its chromophore properties. On the other hand, the small amount of energy that is required to remove electrons makes the carotenoids highly effective antioxidants, although if they act as such then they lose their colorant properties and possibly their therapeutic properties also (Sweeney and Marsh, 1970).

Recent studies dealt with the effect of singlet oxygen and reaction media on the rate and kinetic of carotenoid degradation by light (Goldman et al. 1983, Pesek and Warthesen 1989, Pesek et al. 1990, Minguez-Mosquere and Jaren-Galan 1995). Depending on reaction media conditions

kinetic of photodegradation of carotenoid either zero-ordered or first ordered.

In aqueous model systems and raw foods of high water content the kinetic of photodegradation is different from that of non-aqueous systems or dried foods. In general the rate of carotenoid degradation in aqueous dispersion systems is influenced by the microenvironment of the pigments and sample matrix characteristics (Pesek and Warthesen, 1988). The authors found that temperature had some influence on light-induced degradation rates. With samples exposed to light at higher temperature generally degrade at a faster rate.

2.2.2 Lipoxygenase-catalyzed cooxidation

Lipoxygenase can be used to bleach carotenoids in wheat flour by a cooxidation reaction that requires the presence of a polyunsaturated fatty acid. The cooxidation activity of lipoxygenases may be source-dependent; lipoxygenases in peas and beans have been reported to have a high cooxidation activity (Grosch et al. 1976) and for soybean and peas the 'type II' isoenzymes have been reported to be more effective at cooxidation than 'type I' isoenzymes (Weber et al. 1974). Recently, a high cooxidation activity towards β-carotene and retinyl acetate have been reported for one of two chickpea 'type II' isoenzymes (Sanz et al. 1994). Purified tomato lipoxygenase has been reported to oxidize β-carotene faster than α-carotene and lutein, whereas lycopene, the main tomato pigment, remained unaffected

(Cabibel et al. 1991). Given the choice of natural substrates available and the occurrence of different isoenzymes in any given source, it is not surprising that there have been a number of reports in the literature relating loss of carotenoids as a whole to lipoxygenase activity. Oxidation by lipoxygenase in non-conventional media has been reported by Pourplanche et al. (1993).

Lipoxygenase have been claimed to catalyze the oxidation of carotenoids and chlorophyll by a free radical mechanism that requires the presence of a polyunsaturated fatty acid. It is possible that the enzyme is an integral part of the system for cooxidation of carotenoids through the involvement of an enzyme pentadienyl radical-complex (Klein et al. 1984, Ludwig et al. 1987 and Regdel et al. 1985). The cooxidation reaction may arise from abstraction of a hydrogen atom from a carotenoid resulting in the formation of a resonance-stabilized radical which can combine with oxygen to produce carbonyl compounds (Klein et al. 1985). Further products may arise either by decomposition of the radicals or by condensation to form dimmers or higher polymers. However, little is known of the chemistry of the degradation products (Cabibel et al. 1991). One potential mechanism involves the leakage of a peroxyl radical from the enzyme that can then attack the carotenoid, presumably at positions adjacent to double bonds. A second possibility is that an enzyme-bound hydroperoxide is the oxidizing species. A third mechanism could be the generation of free radicals in reactions catalyzed by anaerobic cycling of lipoxygenase. Whatever the primary mechanism, the net effect generates carotenoid moieties containing a

free radical center which can then react with oxygen to cleave an adjacent double bond to give two carbonyl fragments. However, the oxidized products generally have not been identified yet. In addition to loss of carotenoid color, such processes, if continued, may lead to the formation of odorous molecules. Some work with tomato lipoxygenase has indicated that cleavage can occur at a number of the double bonds in lycopene and that 6-methyl-5-hepten-2-one is one of the products (Robinson et al. 1995). During the apparent associated oxidation of other substances, including thiol groups and inhibitors, it is possible that free radicals are first dissociated from the enzyme. Concentrations of β-carotene higher than 14 μM inhibited oxidation by chickpea lipoxygenases (Sanz et al. 1994), which may be due to the formation of an irreversible enzyme-β-carotene complex as suggested by Cohen et al. (1985). Endogenous inhibitors of lipoxygenase in plant sources include chlorophyll, β-tocopherol and phenolic compounds. These substances could act as scavengers for release free radicals and it has been suggested by Regdel et al. (1985) that the mechanism for bleaching of chlorophyll differs from that for carotenoids.

2.2.3 Pigment decolorization methods

Few model systems have been elaborated to study rate and kinetic of decolorization of fat-soluble pigments as a function of auto-, light- and/or enzyme-catalyzed oxidation. In most of these methods unsaturated fatty acids

have been incorporated to enhance accelerated oxidative degradation of the pigments.

The widely used models are those basing on spectrophotometric determination of the change in the concentration of individual pigment dissolved in an organic solvent before and after exposure to the oxidizing agent. Carnevale et al. (1979). Studied degradation rate of β-carotene dissolved in light petroleum and exposed to fluorescent light. Loss of β-carotene was calculated from the decrease of absorbency at 450 nm. In their work on photoisomerization of β-carotene O'Neil and Schwartz (1995) exposed all-trans β-carotene (in hexane) to white light for 48 hr and measured the isomerization extent by HPLC technique. By the same model the authors evaluated chlorophyll derivatives as photosensitizers at different concentrations and incubation temperatures.

Non-aqueous and aqueous models of some carotenoids were prepared by Minguez-Mosqnera and Jaren-Czalan (1995) to study kinetics of decolourization of carotenoid pigments in the water free system, the pigments were dissolved in ethanol or cyclohexane and exposed to a white light in a compartment to receive an illuminance of 1000 Ix. The progress of decolorization reaction was monitored spectrophotometrically at 460 nm. For preparation of aqueous model Tween 20 was added to the pigment extract before removing the solvent. An oil in water emulsion was then made by slow addition, with gentle shaking, of deionized water. The suspension was then exposed to the white light for several time intervals.

The kinetic of carotenoid photodegradation in a vegetable juice system has been studied by exposing samples in glass vials to 230 ft-c of light at 4 °C. During an 8-day period, carotenoids have been analyzed by HPLC at selected time intervals (Pesek and Warthesen, 1987).

To a better understanding of carotenoid stability in foods (Pesek and Warthesen, 1988) worked out an aqueous model system basing on aqueous dispersion to 250 ft-c of light at varied times and temperatures to study the kinetics of photodegradation of carotenoids aqueous media.

Low-moisture model systems were the target of some investigations. Chou and Breene (1972) prepared the model system by combining a solution of pure β-carotene (in chloroform) with microcrystalline cellulose (Avicel) and distribution of β-carotene. The oxidative degradation was monitored by spectrophotometric measurement of β-carotene concentration in 1 g of model system after extraction with 10 ml chloroform. Similar models Teixeira Neto et al. (1981) studied oxygen uptake (headspace and entrapped oxygen) and decolorization of β-carotene. The purpose of the study was to correlate between color degradation and oxygen uptake in dehydrated food systems. In the work of Haralampu and Karel (1983), dehydrated sweet potatoes have been used as natural solid support of β-carotene and ascorbic acid. The loss of color was intensively of the model system.

2.3 Antioxidants

To retard/inhibit the described autoxidation reactions various preventive measures are employed in industrial practice. Replacement of ambient oxygen, for example by nitrogen as an inert gas, is one very effective means of stopping oxidative degradation. However, under industrial conditions, oxygen can never be entirely replaced, and so only a reduction in speed of oxidative degradation can be achieved. Oxygen can also be eliminated, at least partially, by vacuum packaging of the foods. In addition, oxidation can be delayed by storage of the food under refrigeration or freezing. The lowering of the temperature at storage conditions reduces the rate of reaction (Burri et al. 1989).

Most of these methods can only be used for a limited range of products. Therefore the most common procedure is the addition of antioxidants, mainly of fat soluble nature. They used to protect oils and fats and lipid-containing foods. Such foods are however, more difficult to stabilize because the lipids are widely dispersed in the foods, have a large surface or have only limited access to antioxidants added during processing.

2.3.1 Tocopherol

Vitamin E is a family of compounds known as tocopherols and tocotrienols, in which α–tocopherol is the major component and the most biologically active form. All four tocopherols and tocotrienols, with the chemical structures given in Figure 2. These redox-type lipids are of

nutritional/physiological and analytical interest. Vitamin E is a fat-soluble, it is the major antioxidant vitamin transported in the bloodstream by the lipid phase of the lipoprotein particles. Vitamin E is found in nature, primarily in cereals (especially wheat germ oil), nuts and vegetable oils (Esterbuauer, 1991).

Vitamins are essential for normal growth and development of the human body and vitamin deficiency often leads to clinical abnormalities (Murcia et al. 1992 and Asghar et al. 1991). Perhaps the best known of the natural antioxidants, are ubiquitously present in plant tissues as a blend of α, β, γ, and δ homologies. Low concentrations of β-tocopherol are claimed to be associated with an increased prevalence of coronary heart disease (Ohrvall et al. 1996).

Their antioxidant activity decreases from delta through alpha. While vitamin E activity increase in the reverse series, with α-tocopherol being the most abundant and active source of vitamin E. Dugan, (1980) indicates that the tocopherols have been found to be effective as antioxidants in a number of products, including bacon, baked goods butterfat, lard, margarine rapeseed oil, and safflower oil, and sunflowers oil.

The importance of vitamin E for protecting the integrity of lipid structures (especially membranes) in vivo is underscored by the finding that it is the only major lipid-soluble, chain-breaking antioxidant that has been found in plasma, red cells, and tissues (Burton et al. 1982 and 1983). This

finding holds true even in the plasma of children with chronic, severe vitamin E deficiency (Ingold et al. 1987 and Burton et al. 1990).

Tocopherols show their greatest potency as antioxidants in animal fats, carotenoids, and vitamin A (Cort et al. 1974).

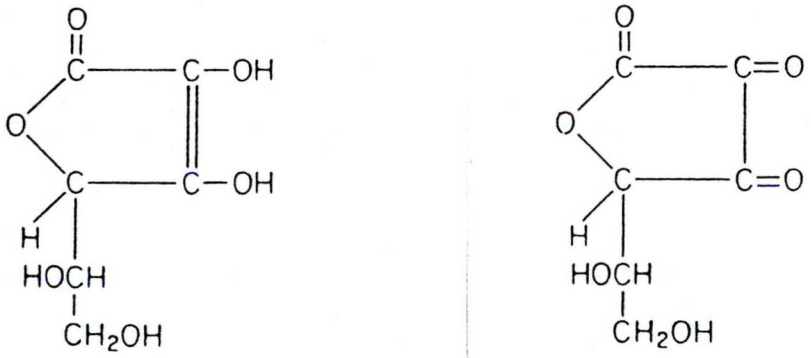
Unsaturated vegetable oils with their inherent tocopherol contents do not benefit much from the addition of tocopherols. The tocopherols are more stabilizing in animal fats than they are in some vegetable oils. To explain this difference in activity, Cort, (1974) compared the antioxidant activities of α-tocopherol, γ-tocopherol, BHA and BHT on oleic acid, which is a constituent of animal fat systems, and linoleic acid, an essential fatty acid obtainable from plant sources. Results showed that the tocopherol were equally or more active than BHA and BHT in oleic acid, the reverse was observed in linoleic acid. Trends similar to those observed with oleic acid were reported in another study involving beef, chicken, and pork fats, thus suggesting the usefulness of tocopherols in animals fats and in vegetable oil with high oleic and low linoleic acid contents.

The susceptibility of vitamin E to oxidation is another important cause of losses during processing and storage. The loss of vitamin E activity can occur either via mechanical loss or by oxidation (Fennema et al. 1985). In its function as a natural antioxidant, the oxidation of vitamin E prevents lipid oxidation, especially of polyunsaturated fatty acids. Losses of vitamin E are therefore associated with its oxidation, as well as overall lipid oxidation (Slover et al. 1972 and Hakansson et al. 1992).

2.3.2 Ascorbic acid

Vitamin C is the major water-soluble antioxidant. It is located in the blood plasma assumed to be able to regenerate oxidized vitamin E which is located in the LDL, thereby permitting the continued inhibition of lipid peroxidation (Frei et al. 1988 and Niki et al. 1987). The newly measured values of vitamin C content in foods by high performance liquid chromatography showed that the most important vitamin C sources are orange/orange juice, grapefruits, tomatoes/tomato juice, potatoes, broccoli and paprika (Sinha et al. 1993).

L-Ascorbic acid is a highly soluble in compound that has both acidic and strong reducing properties. The natural form of vitamin is the L-isomer; the D-isomer has about 10% of the activity of the L-isomer and is added to foods for non-vitamin purposes (Fennema et al. 1985). The chemical structure of ascorbic and dehydroascorbic acid is shown in Figure 3.



L-ascorbic acid *L-dehydroascorbic acid*

Figure 3. Structure of ascorbic acid and dehydroascorbic acid.

Vitamin C also functions as an oxygen scavenger, making it particularly useful in canned or bottled products with a head space of air. About 3.5 mg of ascorbic acid are required to scavenge the oxygen in one cm^3 of head space (Cort et al. 1982). To be active, ascorbic acid requires that the 2- and 3-position be unsubstituted and available for binding with oxygen, much like ascorbyl palmitate. In its removal of oxygen from air or food, ascorbic acid is oxidized to form dehydroascorbic acid thereby asserting its antioxidant action. Dehydroascorbic acid formed as the initial product of oxidation, is biologically active as vitamin C. The mechanisms of degradation are mainly dependent on the effects of pH, metals, oxygen pressure, and water activity (Frankel et al. 1989). Like ascorbyl palmitate, ascorbic acid is more active in retarding rancidity than BHA and BHT but to a lesser degree than TBHQ and propyl gallate in oil systems with high tocopherol contents. Ascorbic acid synergizes the effects of tocopherols.

The instability of L-ascorbic acid, however, has been very well documented and has been demonstrated to be affected by a number of factors. Temperature has been reported to be the most critical factor in preserving L-ascorbic acid (Nagy et al. 1980). In solutions, and even in the pure dry state, L-ascorbic acid has been demonstrated to degrade with time (Kennedy et al. 1989a). Other factors and constituents of juice such as pH, heavy metals like copper and iron, oxygen, amino acids and sugar and type of packaging have been shown to affect L-ascorbic acid stability (Trammell et al. 1986, Kanner et al. 1989 and Kennedy et al. 1989b).

Its ability to reduce components may be extended to antioxidants. For instance, ascorbic acid and its sodium salt, sodium ascorbate, are hypothesized to regenerate phenolic antioxidants by contributing hydrogen atoms to phenyl radicals produced by lipid oxidation (Lindsay et al. 1976). In addition, it has been found that tocopherols were recycled form their oxidized states at the expense of ascorbic acid when ascorbic acid was added to the system (Packer et al. 1979). By itself, ascorbic acid which is freely water soluble has hardly any antioxidant activity. When used in combination with other antioxidants it functions as a synergist by promoting their antioxidative effects (Cort et al. 1982).

2.3.3 Carotenoids

Of the food colorants, the carotenoids are of special interest, not only because they are responsible for the attractive color of many foods but also because they represent a major dietary source of vitamin A. Carotenoids are pigments of yellow, orange-red color, occurring in both the flora and the fauna. In the leaves of higher plants, carotenoids are found together with chlorophylls. They also occur in other parts of plants (e.g. in roots, fruits) and micro-organisms and in many cases they are the major pigments in the exoskeleton of aquatic and avian species (Sies et al. 1991). They are very widespread and occur naturally in large quantities. Isler et al. (1971) has estimated that over 100,000,000 tons of carotenoids are produced annually by nature.

Carotenoids are a group of mainly lipid-soluble compounds. They are polyene hydrocarbons biosynthesized from eight isoprene units (tetraterpenes) and, correspondingly, have a 40-C skeleton. Other carotenoids are derived by hydrogenation, dehydrogenation and/or cyclization of the basic structure of the 40-C carotenoids. The latter reaction can occur at one or both end groups. About 60 different end groups are known, comprising about 450 known carotenoids, and new ones are continually being reported. Most of the carotenoids reported earlier have a 40C central skeleton, but recently some have been described that contain more than 40 carbons. Figure 4 shows the structure formulas of some carotenoids. They are also named as substituted C40 carotenoids (Belitz et al. 1992, Bauernfeind et al. 1981, Chichester et al. 1972, Isler et al. 1971 and Zakaria et al. 1979).

It has long been demonstrated that two different pathways of carotenoid biosynthesis are distinguishable in higher plants; light-dependent and light-independent. In light-dependent pathways, carotenoids are formed together with the chlorophyll in chloroplasts. The biological role of carotenoids in the chloroplast system is to protect chlorophyll from oxidative damage and to transfer light energy through the interior biomembranes of the chloroplast envelope. The major carotenoids of such a pathway are β-carotene, lutein, violaxanthin and neoxanthin. In some plants such as leafy vegetables, this results in a formation of considerable amounts of β-carotene that makes the products useful for the provision of dietary provitamin A.

The light-independent pathway is initiated immediately after chlorophyll degradation as a function of ripening process in fruits and vegetables. Depending on the type, horticultural crops differ substantially in the content and composition of carotenoid pigment. For instance carrot, tomato and apricot distribute mainly oxygen non-containing carotenes, whereas red pepper, citrus fruits and peach contain, in addition to carotene, fatty acid esters of OH-containing xanthophylls (Biacs and Daood, 1994).

Carotenoids are the most widespread group of naturally occurring pigments in nature present without exception in photosynthetic tissues and occurring with no definite pattern in non-photosynthetic tissues such as roots, flowers, petals, seeds and fruits. They are also found in micro-organisms and in many cases they are the major pigments in the exoskeleton of aquatic and avian species (Bauernfeind, 1981).

In green plant tissues, where chloroplasts are locating, carotenoids are synthesized together with other type of fat-soluble pigments "chlorophylls". The name "chlorophyll" was originally intended to describe those green pigments involved in the photosynthesis of higher plants, the most important biological process occurring in photosynthetic biomembranes. Because of their widespread occurrence in a variety of vegetable, fruits, legumes and other plant products, chlorophylls play a vital role in the acceptability of food commodities. For instance, a radical shift in the color of food, even though accompanied by no change in flavor of texture, can make the food completely unacceptable to consumers.

Chlorophylls occur in the membranes of chloroplasts, the organelles that hold chlorophylls close to the cell wall, and carry out photosynthesis in plant cells. In the biosphere at least nine types of chlorophylls can be identified; but only two types, chlorophylls a and b, which occur in the approximate ratio of 3:1 in higher plants are of interest from the viewpoints of food science and technology. The other types such as chlorophylls c, d, and e, bacteriochlorophylls a and b and chlorobium chlorophylls occur only in microorganism and algal biomass.

Leafy vegetables, green peppers, cucumbers and unripe fruits are the main sources of chlorophylls in edible foods. Chemically, chlorophylls are complex organic molecules composed of four pyrrole rings. The central magnesium atom is bonded covalently to two nitrogen atoms. Additionally, two more coordinate bonds are formed when the other nitrogen atoms share two electrons with the magnesium. The chemical nature of pyrrole rings enables chlorophyll to associate readily with lipophilic components such as phospholipids as well as membrane proteins. Association of chlorophyll with neutral lipids and carotenoid pigments is facilitated by phytol. The phytol side-chain is therefore responsible for the hydrophobicty of chlorophylls and their derivatives. Figure 5 shows the structure formulas of chlorophylls a and b.

2.4. Biological role of antioxidant vitamins

Antioxidants are substances with the function to retard rancidity by inhibiting the autoxidation reactions. There are chain-breaking antioxidants (AH) which can interfere during the initiation and propagation reactions (reaction 7, 8 and 9).

$$ROO^{\bullet} + AH \equiv\equiv ROOH + A^{\bullet} \qquad (7)$$

$$A^{\bullet} + ROO^{\bullet} \longrightarrow \text{Nonradical products} \qquad (8)$$

$$A^{\bullet} + A^{\bullet} \longrightarrow \text{Nonradical products} \qquad (9)$$

Chain-breaking antioxidants include phenols with bulky alkyle substituents designed to produce antioxidant radicals ($A^{\bullet}$) that are too unreactive to propagate the chain. Examples include: tocopherols, butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), butylated hydroquinone (TBHQ), and propyl gallate (PG). To be effective this type of antioxidant must be able to compete with the substrate for the chain-carrying peroxyl radicals, normally present in the highest concentrations in the system (Scott et al. 1985).

Another group of antioxidants (Q) behaves as chain-breaking electron acceptors by trapping alkyl radicals, provided they can compete with oxygen for alkyl radicals (reaction 10) (Scott et al. 1985).

$$R^{\bullet} + Q \longrightarrow RQ \qquad (10)$$

Biological electron-acceptor antioxidants include α-tocopheroquinone, the oxidation product of α-tocopherol, vitamin K and ubiquinone. These compounds are effective if they can compete with oxygen in trapping alkyl radicals, especially at the low oxygen pressures prevailing in healthy tissue.

Other types of antioxidants include: (a) preventive antioxidants, such as citric, phosphoric and ascorbic acids, act as metal inactivators by removing or deactivating metal ions which catalyze the decomposition of hydroperoxides; (b) peroxide destroyers, reduce hydroperoxides into stable alcohols by nonradical processes. Examples include sulfur compounds, phosphites, which unfortunately cannot be used in foods; (c) UV deactivators, absorb irradiation without formation of radicals. Examples are pigments like carbon black, phenyl salicylate and α-hydroxybenzophenone (Bailey et al. 1962). It has been hypothesized that dietary antioxidants such as vitamin C and β-carotene reduce risk of many cancers by inhibiting the formation of carcinogenic nitrosamines, scavenging and quenching reactive oxygen molecules, thereby protecting DNA from oxidation damages and enhancing immune functions (Byers et al. 1992, Block et al. 1992 and Halliwell, 1994). It has long been known that α-tocopherol and ascorbic acid are two of the most effective *in-vivo* antioxidants (Bendich et al. 1989 and Krinsky et al. 1989).

It has been reported that a diet higher in vitamin C by the amount in one or two oranges per day and higher in β-carotene by the amount in one or two carrots per day was associated with a 31% lower risk of death during 24

years of follow-up in a cohort of employed, middle-aged men (Pandey et al. 1995).

Synergism is the process by which the antioxidant effect of multicomponent systems is reinforced. Significant synergism is generally observed when chain-breaking antioxidants are used together with preventive antioxidants or peroxide destroyers because they suppress both initiation and propagation. The admixture of free radical acceptors and reducing agents causes synergism by the regeneration and recycling of the antioxidant (Ingold et al. 1961).

2.4.1. Tocopherol (vitamin E)

The importance of vitamin E for protecting the integrity of lipid structures (especially membranes) in vivo is underscored by the finding that it is the only major lipid-soluble, chain breaking antioxidant that has been found in plasma, red cells and tissues (Cheesman et al. 1984, 1988).

Because α-tocopherol can compete for peroxy radicals much faster than can polyunsaturated fatty acids, a small amount of α-tocopherol is able to protect a large amount of polyunsaturated fat. Concentrations of α-tocopherol in biological membranes are approximately one part per 1000 lipid molecules (Burton et al. 1983). Demonstration of the effectiveness of vitamin E in lessening the effects of lipid peroxidation in living systems are rare and difficult to obtain. However, it has been reported that pentane, a minor product released during the peroxidation of polyunsaturated fat, is

reduced in the breath of human supplemented with vitamin E (Lemoyne et al. 1987, 1988). This strongly suggests that vitamin E prevents the peroxidation of polyunsaturated fat in vivo.

The contribution of α-tocopherol as a prooxidant has been proposed recently (Bowry et al. 1992, 1993 and Ingold et al. 1993). That is, α-tocopherol which resides at or near the surface of membrane reacts more rapidly with attacking aqueous radicals than the lipid to give α-tocopheroxyl radical, which attacks lipid to give lipid radical to initiate the chain reaction. Thus, α-tocopherol is assumed to behave as a chain-carrier and enhance the efficiency of lipid radical formation. The overall potency of α-tocopherol as an antioxidant depends on the fate of α-tocopheroxyl, but it is not clear at present how such a prooxidant action of α-tocopherol is important in the membranes and lipoprotein in vivo where the vertical motion of α-tocopherol is restricted and where the reductants may well react with α-tocopheroxyl radical to generate α-tocopherol (Niki, 1987, 1993 and Burton et al. 1993).

2.4.2. Ascorbic acid (vitamin C)

This organic acid has a complex multi-function that has led to much confusion in the literature as to whether it acts as an antioxidant, a prooxidant, a metal chelator or a reducing agent. A combination of these effects may prevail in many food applications. In aqueous media, ascorbic acid is a powerful prooxidant in the presence of metal ions. Metals reduced

by ascorbic acid are more catalytically active state (Porter et al. 1980). In the absence of metals, ascorbic acid acts as an antioxidant, especially at higher concentrations (Cort et al. 1982). In nonaqueous media the antioxidant effects of ascorbic acid and esters are regarded as not pronounced (Porter et al. 1980).

There is a large body of literature on the stability and oxidative degradation of ascorbic acid in foods (Tannenbaum et al. 1976 and Gregory, 1985). Dehydroascorbic acid formed as the initial product of oxidation, is biologically active as vitamin C. The mechanisms of degradation are mainly dependent on the effects of pH, metals, oxygen pressure and water activity (Frankel et al. 1989).

In vivo and in vitro studies have shown amply a statistically significant effect of vitamin C in protecting lipids from oxidation (Simon et al. 1992). Furthermore, it has been reported that people with low vitamin C levels have higher amounts of lipid peroxides in plasma than do people with high vitamin levels (Hemila et al. 1992). Accordingly, vitamin C may also affect cholesterol metabolism through the antioxidant effect. Ascorbyl palmitate and other esters are more useful as inhibitors in oils because they are more soluble than the free acid (Cort et al. 1982).

2.4.3. Carotenoids (provitamin A)

Carotenoids protects lipid against photosensitized oxidation by acting as quenchers, which interfere with the activation of triplet oxygen into

singlet oxygen. β-carotene is regarded as an extremely effective quencher of singlet oxygen. It is estimated that at least 1,000 molecules of singlet oxygen are quenched for each molecule of β-carotene consumed (Foote et al. 1970). In addition to its ability to accept singlet oxygen energy, β-carotene can also inhibit lipid peroxidation by either trapping free radicals (Krinsky et al. 1982), or by acting as a competitive substrate by delaying initiation and propagation reactions (Ames et al. 1983, 1987 and Simic et al. 1980).

It is well known fact that some dietary carotenoids, when absorbed, are converted to vitamin A in the intestine, a process which is under homeostatic control. β-carotene is transported by the chylomicrons to the liver (Biesalski et al. 1994). Non-provitamin A carotenoids such as lutein, lycopene, neoxanthin, violaxanthin have been found in blood and different organs of animal and human body revealing the high biological activity of such compounds.

β-carotene is a lipid-soluble and is concentrated in circulating lipoprotein and atherosclerotic plaques. In humans, LDLs appear to be the major carrier of the serum carotenoids and presumably play an important role in the transport of carotenoids from one organ to another (Chen et al. 1974 and Cornwell et al. 1962).

Chemically, β-carotene is much less reactive toward peroxyl radical than α-tocopherol but may act as a moderate radical scavenging antioxidant, especially in the lipophilic domain of the membranes at low oxygen

concentration. It may also be possible that β-carotene and α-tocopherol act cooperatively as an antioxidant in the membranes and lipoproteins (Tsuchihashi et al. 1995).

The peroxyl radical-trapping activity of β-carotene and possibly other carotenoids is dependent on the partial pressure of oxygen applied (Burton and Ingold, 1984). It is less efficient under conditions of air, but becomes a good peroxyl radical trap at the low pO_2 (Burton and Ingold, 1984, Stocker et al. 1987b) that prevails in biological tissues. At the very low pO_2 or 4 torr, β-carotene inhibited adriamycin-enhanced microsomal lipid peroxidation even more efficiently than α-tocopherol, while the antioxidant protection observed with retinol did not show a dependency on oxygen concentration (Vile and Winterbourn, 1988). Like carotenoids, membrane-bound bilirubin scavenges peroxyl radicals also more efficiently under low pO_2 where it can become as efficient as α-tocopherol (Stocker et al. 1987b). These observations are of potential physiological significance as the oxygen pressure in human arterial and venous blood is around 68-93 and 40-61 torr, respectively (Gibbs et al. 1942, Lentner, 1984), and decreases further within tissues.

A photoprotective mechanism of β-carotene in skin involving scavenging of singlet oxygen was suggested. Although clinical effectiveness of β-carotene in the treatment of erythropoietic protoporphyria is suggested

by several other case reports, controlled trials have not been performed (Pollitt, 1975).

2.5 Trace metal pollution and antioxidant vitamins in foods

Trace metals are that group of elements that are of environmental importance, usually occurring in minute quantities, and whose presence in the soil-plant system is primarily via human activities. The content of available harmful elements in soil is increased by some orders of magnitude in urban-industrial areas or through addition to the soils of sewage sludge, swine manure, and some pesticides with high metal content (Aderiano et al. 1986).

Composition and quality of the soil may affect qualitative changes in the plants growing on these soils and in the humans and animals eating the plants. According to the literature data (Purves et al. 1985 and Fergusson et al. 1991), the concentration of some heavy metals increased by orders of magnitude in the blood, urine, hair and other tissues of urban populations. Moreover, soil contamination by toxic/harmful metals represents one form of chemical burden on the environment and it exerts numerous sanitary, economical and ecological after-effects.

A spin off area of toxicity research is plant adaptation to high levels of metals in soils. Depending on types and genotypes, plants show different responses to heavy and toxic metals (Brown et al. 1961 and Aderiano et al. 1986). Growth, yield and sometimes chemical composition of the edible part

can be taken as indices of plant adaptation or to metal contamination in the soils (Biacs et al. 1995).

It is well established that micronutrients interact with organic compounds in the metabolism as metal-linked complexes. Interference of micronutrients with metabolic pathways of a living plant can reduce the biosynthesis of important dietary nutrients in plants. Of the vital nutrients in human diet, carotenoids, tocopherols and ascorbic acid have aroused greater interest lately most likely due to their role in prevention of many diseases as previously mentioned (Moon et al. 1988).

In accordance with an earlier investigation (Kádár et al. 1992), mainly tuber and root crops, leafy vegetables and fodder plants are threatened by the contaminants originating from traffic, industry and heavy polluted urban areas.

3. MATERIALS AND METHODS

3.1 Materials

Fresh fruits of spice red pepper (*Capsicum annum*) were obtained from Research Station of Spice Red Pepper, Kalocsa and Spice Red Pepper Processing RT, Szeged.

Paprika powders were prepared by grinding the semi-final (dried fruits) product by a coffee mill to pass a 20 mesh sieves (approx. 0.5 μ particles).

Tomato fruits (Lycopersicon esculentum) from different cultivars and at different stages of ripening were from the College of Horticulture, Kecskemét.

Spinach (Spinalia oleracea var. Matador) was obtained from the research experimental fields of the Research institute of Soil and Agrochemistry (TAKI), Nagyhörcsök, where experiments on trace elements are carried out. Spinach leaves were harvested from at least 20 plants from each trace element treatment, thoroughly mixed and stored at -20 when not in use. The design of trace element experiment is shown in Table 1. Parsley was obtained from the local markets of Budapest.

For each fruit and vegetable tested 3-4 samples from the edible part were taken. All samples were stored at -20 °C in vacuumed nylon sacs when not immediately analysed. To study storage stability of ground red pepper, the samples were packaged in nylon sacs and stored in a locker at ambient conditions for 120 days.

Analytical and HPLC grade organic solvents (carbon tetrachloride, methanol, benzene, petroleum ether, acetonitrile, isoprpanol, chloroform,

hexane and absolute alcohol) and analytical grade chemicals such as metaphosphoric acid, potassium hydroxide, sodium dihydrogen phosphate, sodium carbonate and sodium sulfate anhydrous were purchased from Reanal Rt (Budapest). Standard tocopherols (99% pure), carotenes (96% pure), linoleic acid, Tween-20 and organic acids (99.9% pure) and purified Soya lipoxygenase were purchased from Sigma (St. Louis, Mo).

3.2 Analysis of carotenoids

3.2.1 Extraction of carotenoids

The carotenoids were extracted by the method of Daood et al. (1987). Fresh fruit samples were cut into small pieces and deseeded. Three-five gram samples, in duplicate, were taken, disintegrated with quartz sand in mortar with pestle and mixed with 20 ml methanol. The mixture was mixed with 60 ml carbon tetrachloride-methanol 3:1 and mechanically shacked for 20 min. The CCl_4 fraction was separated from the aqueous phase in a separatory funnel and dried over anhydrous sodium sulfate. The filtrate was evaporated to dryness in a vacuum rotatory evaporator at 30-40 °C.

The pigments of ground red pepper samples were extracted by shaking 0.25 gram of the powder with 50 ml of CCl_4-benzene-methanol 2:1:1 in a mechanical shaker for 20 min. and then filtered. The filtrate was taken and the solvent was evaporated under vacuum in a rotatory evaporator at 30 °C (Daood et al. 1989).

3.2.2 Preinjection preparation

Solid material of extracted pigments was dissolved in 2 ml of chloroform and the volume was then completed to 5 ml with the HPLC eluent. In case of high pigment concentration 2-3 time dilution was done with the HPLC eluent.

3.2.3 HPLC determination of carotenoids

A Beckman series of liquid chromatograph consisting of a Model 114 solvent delivery pump, a Model 421 controller and a Model 165 variable wavelength UV/visible detector was applied. The detector signals were recorded by a Model C-R2A. Shimadzu integrator. To study spectral chracteristics of the individual carotenoids a Waters model photodiode-array detector was used. The data were stored and processed by means of NEC.APCIV power meta 2 IBM computing system. The absorption spectra of carotenoids were recorded between 190 and 700 nm at the ratio of 2 spectra/sec.

Separation of carotenoids was performed on columns (25 cm length x 4.6 mm i.d.) packed with lichrosorb C-18 of either 10μm or 6μm particles. The mobile phase was acetonitrile-isopropanol-methanol-water (39:52:6:4) and the flow rate was between 0.9 and 1.2 ml/min. (Biacs and Daood, 1994).

3.2.4 Identification of carotenoids

The identification of the peaks was done by comparing their retention times and spectra with those of authentic standards according to Biacs et al. (1994). The quantification of carotenoids was carried out by comparing the areas of their peaks with that of pure β-carotene standard (Sigma, St. Lo. USA). Identification of cis isomers of carotenoids was based on the appearance of extra maxima between 320 and 360 nm in the absorption spectrum of the individual peaks (Chandler et al. 1987). As for chlorophyll type pigments existing in green vegetables together with carotenoids, the identification was based on retention times and spectral characteristics compared with authentic standards prepared by TLC according to Daood et al. (1989). The extracts of leafy vegetables were separated on cellulose TLC. The bands were identified by chemical tests, UV-visible spectrum and measuring of retention factors.

3.3 Analysis of tocopherols

3.3.1 Extraction of tocopherols

The tocopherols were extracted by the same method used for extraction of carotenoids (3.2.1).

3.3.2 Saponification

The extracted lipid fraction was saponified by refluxing with 4 ml of 30% methanolic KOH for 35 min. at the boiling point of methanol in the presence of 0.5 gram ascorbic acid as antioxidant. Following cooling the flask under running tap and addition of 20 ml salted water, the tocopherol isomers were extracted twice with 40 ml petroleum ether in a separatory funnel. The ether fractions were collected, washed twice with 50 ml distilled water and dried over Na_2SO_4. The solvent was evaporated under vacuum at 30 °C and the residues were redissolved in 5 ml of HPLC grade hexane.

3.3.3 HPLC determination of tocopherols

A Beckman Model 114 M isocratic pump, a Shimadzu fluorometric detector, and Shimadzu C-R3A or Waters-740 Data Model integrators.
The separation of tocopherols was performed on column (25 cm length x 4.6 mm i.d.) packed with lichrosorb, the mobile phase was n-hexane-ethanol 99.5:0.5, flow rate was 1.2 ml/min. and the detection wave length used was 290-320 nm (Speek et al. 1985).

3.3.4 Identification of tocopherols

For peak identification the retention times and maximum absorption spectra of tocopherol were compared with those standard materials (Sigma, USA) which were used also quantification.

3.4 Analysis of vitamin C and organic acids

3.4.1 Extraction

For extraction of vitamin C and organic acids the method used by Daood et al. (1994) was used. Three-five grams of fresh fruit sample were disintegrated in a crucible mortar with quartz sand. The macerate was then mixed with 50 ml of 2% metaphosphoric acid and transferred to a conical flask. Following mechanical shaking for 15 min., the mixture was filtered through a Rudfilter MN 640 d filter paper (Macherey-Nagel; Germany) to obtain clear extract that were kept at-20 °C until analysis.

In the case of powder samples, organic acids were extracted from 0.25 gram powder by shaking with 30 ml of 2% metaphosphoric acid following the procedure used for fresh samples.

3.4.2 HPLC determination of organic acids

A Beckman series of liquid chromatograph consisting of a model 114 solvent delivery pump, a Model 421 controller and a Model 165 variable wavelength UV/visible detector. The detector signals were recorded a Model C-R2A Shimadzu Integrator, was used. To record spectrum of ascorbic acid a Waters model 990 photodiode-array detector was used. The range of detection wavelength was 190-340 nm.

The isocratic mobile phase was prepared by adding 1.0 ml of 20% tetrabutyl ammonium hydroxide and 30 ml of methanol to 970 ml of 0.01 M

potassium dihydrogen phosphate solution. The pH was adjusted to 2.75 using 85% phosphoric acid. The flow rate was 1.0 ml/min. The separation was performed on ODS-2 10 μm column (25 cm x 4.6 mm i.d). The injection volume was 20 μl.

3.4.3 Identification of organic acids

Chromatographic peaks were identified by comparing both retention time and absorbance spectra with those of standards (Fluka, Switzerland). Co-chromatography of the standard with the samples was also applied to identify peaks with close retention times.

3.5. Effect of elements treatment on antioxidants contents of spinach

3.5.1 Experimental design

The soil of the experimental station (Nagyhörcsök) is a calcareous loamy cherrnozem with about 25% clay, developed on loess. In its plowed layer it contains humus and $CaCO_3$ in about 3% and 5%, respectively. To ensure a sufficient macronutrient supply in the whole experiment, 100 kg/ha N, P_2O_5 and K_2O were given yearly. Ten elements (Cr, Mo, Pb, Se, As, Cd, Cu, Hg, Sr and Zn) were studied. The elements were applied to the soil in 1991 at five levels (0, 30, 90, 270 and 810 kg/ha) and the treatments were arranged in a split-plot design with 2 replications. The area of each plot was 21 m^2.

3.5.2 Determination of microelements

Half g of dried spinach leaves were digested in Teflon bombs with 5 ml nitric acid (65%) overnight. Then 2 ml H_2O_2 (30%) were added and the mixture were placed in an oven at 110 °C for 4 hours. After cooling, the volume was completed to 25 ml with bidestilled water and the solution was filtered through filter paper (589 Blue Ribbon ashless, Scheicher and Schuell, Germany). The analysis of the microelement content was carried out by ICP (Inductively Coupled Plasma, model JY-24, Joben Yvon, France). The elements determined and the wavelengths used were: As (153.699), Sr (181.978), Hg (194.167),), Se (196.03), Mo (202.438), Cr (205.552), Zn (213.856), Pb (220.353), Cu (324.654) and Cd (228.438). The gas (argon) flow rates were: carrier gas 12 l/min, auxiliary gas 0.4 l/min, nebulizator gas 0.4 l/min. The voltage power was 1.1 kW. The Spex multielement Plasma Standard (Spex, USA) was use as standard.

3.6 Model system for *in- vitro* interaction

3.6.1 Aqueous system (LOX-catalyzed reaction)

For studying the stability of different pigments towards LOX-catalyzed oxidation reactions, the carotenoids and chlorophylls were extracted from the different fruit samples with 2:1 (v/v) carbon-tetrachloride and methanol as described previously (Daood et al. 1987). 50 μL portions of both Tween-20 and linoleic acid (needed to give maximum velocity of LOX as reported by

Pourplanche et al. 1993) were added to solid materials of extracted pigments. The mixture was dissolved in 3 ml of chloroform, which then evaporated under vacuum. The residues were suspended in 18 ml buffer of pH 9 (0.05 N disodium hydrogen phosphate) with the help of ultrasonication. Tocopherol (in the form of tocopherol acetate) and ascorbic acid were added at different concentrations to the pigment suspension. The suspension was divided into 3 aliquots in small flasks and vibrated with electric vibrator for 1 minute to insert sufficient amount of air in the mixture. The cooxidation reaction was induced by adding 100 µl of freshly prepared solution of soya lipoxygenase in phosphate buffer pH 9 (10 mg in 4 ml). The pigments were extracted from the mixture in a time intervals of 0, 30, 60 and 120 minutes. The extraction of pigment was carried out by adding 20 ml methanol followed by shaking with a mixture of CCl_4 : methanol (60:40). The other steps as mentioned in 3.2.1 and 3.2.2 (Biacs et al. 1989).

3.6.2 Non-aqueous system (photooxidation)

The extracted pigments were dissolved in 18 ml carbon-tetrachloride, 50 µl of linoleic acid and 50 µl of Tween -20 were added and dispersed by ultrasonic device (Tesla, Czech. Republic). α- and δ-Tocopherol and ascorbic acid (in the form of ascorbyl palmitate to improve its solubility) were added. Solutions of pigments in carbon tetra-chloride (6 ml each) were put in small flask to give a layer of about 2 mm deep. The flasks were placed in a custom-built light chamber and exposed to a light from 40 W fluorescent

lamp that gives an intensity of about 3000 lx at the surface of the samples from a distance of 17.5 cm. To eliminate other source of light the solutions and the lamp were inside the light chamber. With threefold repetition of exposure in sets, the rate of pigment lose was measured after 0, 30, 45, 60, 90 and 120 minutes intervals. The solution was taken and solvent evaporated under vacuum by rotatory evaporator to dryness. The residues were then redissolved in 2 ml chloroform and 8 ml HPLC eluent to be ready for HPLC analysis.

3.7 Statistical analysis

One-factor analysis of variance (ANOVA) was used to estimate degree of significance between different cultivars of some vegetables while multi-factor ANOVA was used to analyse data from the experiments on heavy and toxic metals with several doses.

3.8 Items and definitions

- Percentage retention of the individual pigment was calculated according to:

$$\% \text{ retention} = \frac{\text{peak area at T1}}{\text{peak area at T0}} \times 100$$

when T1 and T0 are the time interval of oxidation.

- Performance of antioxidant function toward oxidation of pigments was expressed as the difference in retention between treated and untreated samples which was calculated using the following formula:

$$\Delta \text{ retention } (\%) == \%\text{ retention }_{\text{Ant.}} - \%\text{ retention }_{\text{Cont.}}$$

- ***Oxidation Prevention Coefficient*** $[\varepsilon^{0.1mg/ml}]$***:*** increase in % retention of a pigment by the system as a function of addition of 0.1 mg/ml antioxidant.

$$[\varepsilon^{0.1mg/ml}] = \frac{[\%\text{ retention }_{\text{Ant.}} - \%\text{ retention }_{\text{Cont.}}] \times 0.1}{\text{antioxidant concentration mg/ml}}$$

This type of evaluation is elaborated in the Lipid Laboratory (KEKI) especially for this work. In the literature % retention has widely been used to express efficiency of natural or synthetic antioxidants in prevention of color degradation.

4. RESULTS AND DISCUSSION

4.1 HPLC determination of antioxidant vitamins

4.1.1. HPLC analysis of tocopherol

Table 2 shows the data of five measurements of antioxidant vitamins using the same fresh fruits or ground paprikas. Values of 5.5, 5.1 and 2.8 of C.V.% for β-carotene, α-tocopherol and ascorbic acid, respectively, were obtained when fresh fruits were analyzed, whereas lower values were recorded for the same vitamins when determined in ground paprika.

Normal phase chromatography gave an excellent separation of tocopherol analogous (α-, β-, γ- and δ-) and some derivatives of ubiquinone by one step isocratic elution. Application of fluorescence detection allowed the monitoring of not only tocopherols but also ubiquinone, which are regarded as effective fat-soluble antioxidants. This could not be achieved by UV detection, which is well known to be substantially less sensitive than the fluorescence detection.

Factors most likely to affect composition and content of fat-soluble antioxidants in plant products may include type, maturity stage and post-harvest treatments.

Figure 6 shows the HPLC separation of tocopherol analogues from spice red pepper. The method applied gave a good separation of α-, β- and γ-tocopherols with their triene derivatives. α-Tocopherol was found to be the major analogue in the extract of spice red pepper peel with minor quantities of β- and γ- analogue. This profile changes when the product contains

considerable amounts of seeds which distribute γ-tocopherol as the major fat-soluble antioxidant.

The HPLC separation of tocopherols extracted from ripe tomato fruits was studied. Similar profile could be obtained, but with higher level of γ-tocopherol than that found in red pepper extract.

In parsley extract, α-Tocopherol was abundant antioxidant with δ-analogue as the minor component.

4.1.2. HPLC analysis of carotenoids

The diversity of carotenoid extract of some plant products such as tomato and paprika makes it difficult to separate the individual carotenoids by one-step procedure. However, the HPLC method elaborated by Biacs and Daood, (1994) provided a good isocratic separation of carotenoids from tomato and spice red pepper.

Figure 7 shows the carotenoid composition of spice red pepper extract. The high diversity of such an extract is due to the presence of four carotenoid classes: (1) free xanthophylls, (2) monoesters, (3) carotenes and (4) diesters. The method used allowed the quantitative and qualitative analysis of about 42 carotenoid compounds without saponification and that has been used by some authors (Matus et al. 1981, Chandler et al. 1987 and Almela et al. 1991) to simplify the sample. Similar separation has been achieved by other authors, only with gradient elution (Matus et al. 1991 and Deli et al. 1992).

Shown in Figure 8 the separation profile of tomato pigment. The pigment was fractionated into 14 components of which lutein, β-cryptoxanthin, lycoxanthin, lycopene, neolycopene, γ-carotene and β-carotene were the major carotenoids with lycopene being the main red-colored one accounting for more 75% of the total carotenoid content.

A good separation of parsley pigments was obtained as shown in Figure 9. Simultaneous detection of both carotenoids and chlorophylls was carried out at 440 nm. In addition to chlorophyll derivatives the profile contained yellow carotenoids such as polar xanthophylls, lutein and β-carotene isomers. When monitored at 660 nm the HPLC profile showed only chlorophylls that consisted of phytyl free chlorophyllides and pheophorbides followed by chlorophyll b, chlorophyll a, pheophytin b and pheophytin a. In green vegetables such as parsley, chlorophylls account for more than 60% of the total pigments, while β-carotene about 16% as shown in the figure.

Among carotenoids separated by HPLC from fruits and vegetables β-carotene, lycopene, lutein and creptoxanthin are of special interest because they are the only ones that have antioxidant properties in human and animal body (Burton et al. 1984). Furthermore, only the all trans form of carotenoids which has been reported to be the most biologically active analogue of carotenoid and its conversion to cis- form reduces substantially its antioxidant activity (Sies, 1991).

4.1.3. HPLC analysis of vitamin C

By ion-pair chromatographic ascorbic acid could be separated from its oxidized form (dehydroascorbic acid) and other interfering materials in the extract of spice red pepper, tomato and parsley as shown in Figure 10. To check whether ascorbic acid peak contains some interfering materials, a peak purity displayed by the chromatographic software denoted that an organic acid having absorption maxima of 190 nm and 208 nm overlapped with ascorbic acid. Fortunately such interfering materials did not affect quantitation of ascorbic acid since their absorption at the monitoring wavelength used (245 nm) is negligible, and therefore, it can be said that the detection, by this method, of vitamin C is highly accurate.

4.2. Changes in antioxidant vitamins during ripening

During ripening process many vegetables undergo characteristic changes that, in general, lead to an increase in the nutritive value and consumer acceptance. However, some important micronutrients tend to decrease when the fruits become fully ripe. This depends upon the mechanism of ripeness process (climacteric or non-climacteric).

4.2.1. In paprika

Color ripening of paprika fruits can be characterized by five stages as shown in Figure 11. At the first stage (green fruits) small amounts of

ascorbic acid, tocopherol and β-carotene could be estimated in SZ-20 cultivar, with ascorbic acid being the main antioxidant vitamin. The concentration of β-carotene formed through light-dependent carotenoid biosynthesis in green tissues depends to a great extent on the nature and photosynthetic activity of the chloroplasts.

Because of climacteric ripening of paprika fruit, ascorbic acid tended to increase markedly while slower increases were seen for tocopherol and β-carotene. During ripening of ascorbic acid there was a steady-state between stages 2 and 4 followed by a decline in its concentration at the post-ripeness (stage 6). When the fruit lost more than 50% of its water content and both lipophilic and hydrophilic systems were exposed to high oxidation stress, both ascorbic acid and tocopherol performed antioxidant functions. One of the most important ripening processes in climacteric fruits and vegetables is the induction of light-independent carotenoid biosynthesis which often occurs simultaneously with the destruction of the chloroplast system (chlorophyll destruction). The concentration of β-carotene, a major component of paprika pigment, increased gradually at the first stages and to a high level at the last stages of the progressive ripening. During the ripening and post-ripening stages no decrease was recorded in β-carotene content, revealing that it is well protected from oxidative degradation.

In case of Km-622 cultivar there is high similarity in the change of tocopherol and carotenoids, but ascorbic acid showed different manner. For instance its concentration was much higher in green fruit than that estimated

for SZ-20 cultivar at the same stage of ripening (Figure 12). The maximum concentration of vitamin C was at color break-1 and then declined in accord with the advance of progressive ripening to reach a steady-state at overripeness. As concern carotenoids and tocopherol no substantial variation was observed in their trend during ripening of Km-622 cultivar from that of SZ-20 cultivar.

4.2.2. In tomato

To characterize the dynamics of ripeness of tomato fruits of Floriset cultivar, they were harvested at four ripening stages; green, color break-1 (yellow), color break-II (pink) and red. Figure 13 shows the changes occurred on the vitamin C content of tomato fruits during ripening stages. The maximum concentration of ascorbic acid was estimated in tomato fruits that turned yellow in color (color break-1), but the advanced ripeness caused ascorbic acid content to decrease, most likely due to its antioxidant function when the ripening cells absorb high amounts of oxygen as a result of increasing rate of cell respiration, the characteristic physiological change in climacteric fruits and vegetables at ripeness (Turk et al. 1993).

Ripening of tomato is characterized by destruction of chlorophyll type pigments and accumulation of carotenoids (yellow and red). The advance of climacteric ripening brings about rapid formation of lycopene and β-carotene as the major constituents with lycoxanthin and lutein as the minor ones. In the cultivar used, lycopene comprised about 85% of the total pigments, while

β-carotene accounted for 9-11% of the total pigments. As a function of increasing rate of carotenogenesis, the total carotenoid content of tomato was increasing proportionally to the progressive ripeness (Figure 14).

As for β-carotene, its concentration increased proportionally to the advanced ripeness in accordance with the rapid accumulation of red pigments (fruit coloration). Such change tendency does not agree with that observed by Czinkotai and co-workers (1987) who found that β-carotene approached its maximum level in yellow colored fruits of ventura cultivar (tomato for processing) and then declined. This variation is probably due to the effect of some varietal factors on the carotenogenesis in tomato fruit.

The change in tocopherol analogues as a function of climacteric ripeness is depicted in Figure 15. α- and β-tocopherol increased proportionally to the advanced ripeness, while γ-tocopherol approached its maximum level at color break-I stage (yellow fruits) then declined showing similar change to that observed with ascorbic acid. The manner of γ-tocopherol and ascorbic acid revealed their important role as first oxidation barrier in the lipophilic and hydrophilic phases of the foods. Despite the high antioxidative activity of γ-tocopherol in the food systems it is of no importance from the nutritional point of view because of being biologically inactive as vitamin E in human body. As being highly reactive antioxidant, it is very important in prevention of foods against oxidative deterioration, taking place during ripeness, processing and storage of food stuffs.

4.3. Interaction between antioxidant vitamins in dried food (Red pepper)

Usually paprika is ground to a powder of about 0.5 μm particles. This increases to a great extent the surface area and thus enhances lipid oxidation several fold. The detrimental effect of lipid oxidation on the whole biological system can be eliminated if the natural antioxidants are available and able to perform their function.

Figure 16 shows the change in α-tocopherol, ascorbic acid and β-carotene as a function of grinding and storage of ground paprika. Dramatic decrease was noticed in the concentration of both α-tocopherol and ascorbic acid, indicating that they act as the first oxidation barrier. Ground paprika lost 70, 90 and 100% of its α-tocopherol content after 30, 60 and 90 days of ambient storage, respectively. Ascorbic acid content of the same sample decreased to 35, 20 and 10% of the original level after a storage of 30, 60 and 120 day, respectively. β-Carotene, slightly decreased during the first storage period (2 months) but dramatically shifted to low levels during the second half of the storage when the concentrations of α-tocopherol and ascorbic acid were too low to be effective in the antioxidation process.

The behavior of antioxidant vitamins and β-carotene during storage of finely ground paprika was similar to that observed in blood samples by Esterbauer, (1991) who stated that vitamin E provides a first oxidation barrier, while ascorbic acid is needed for its regeneration and the carotenoids function as a second oxidation barrier against lipid oxidation. The role of

β-carotene as a single oxygen quencher in food systems (Yung et al. 1991) and free radical trapper in biological systems of low oxygen pressure (Burton et al. 1992) has been well demonstrated.

In order to study the correlation between the endogenous antioxidant vitamin content and carotenoid retention after a 6 month storage, different paprikas having different tocopherol and ascorbic acid content were ground and stored at ambient temperature is a locker (dark). The antioxidant content of the freshly ground samples were determined and plotted versus color retention (%) after 6-month storage and linear regression analysis was carried out. As shown in Figure 17 positive correlation was obtained with (r) value of 0.938 and 0.969 when ascorbic acid and α-tocopherol concentration in the samples were plotted against retention (%) of carotenoid by the stored ground samples. It was noted that although α-tocopherol content is much less than that of ascorbic acid, the former showed stronger correlation and better antioxidant effect than the later. This is likely due to the high antioxidant capacity of α-tocopherol in non-aqueous or dry systems in which both tocopherol and carotenoid are locating and functioning in the lipophilic phase, while ascorbic acid (hydrophilic) perform its antioxidant function when the water content (water activity) is relatively high. These results confirmed and in full agreement with those obtained by Biacs and co-workers (1989) on stability of paprika powders from different Hungarian cultivars.

4.4. Effect of element stress in soil on spinach leaves

4.4.1. Element treatments and phytotoxicity

Since environmental pollution, from traffic and industry is dramatically increasing content of some toxic metal in soil, will grow up to a very high level some day in future. Therefore it is essentially needed to study which type of plant product can be cultivated with minimal risk and what change on the chemical composition are expected. The design of this experiment was based on conception estimation of the expected level of soil load with the most familiar toxic metal coming from the industrial wastes. The designer of this experiment is the Reaserch Institute for Agricultural and Soil Chemistry (TAKI) in Budapest.

It has been well demonstrated (Biacs et al 1995) that availability of different elements increases as the dose of treatment is raised, thus, assisting in increasing uptake and mobility of the elements via plant roots. Table 3 shows the content of the ten element tested in the leaves of spinach as influenced by different doses. As a consequence of element availability in soil, the concentration of each element increased significantly in spinach leaves. Maximum levels of 765 mg/kg was estimated for Se in plants treated with 90 kg/ha followed by 670.5 mg/kg for Mo in plants grown in soil loaded with 810 kg/ha. It was remarkable that further uptake of Se caused the plant to die out. The lowest level (0.59-1.79 mg/kg) was recorded for Pb content which was not influenced by element load in soil. In case of As and Hg, their

uptake by plant leaves was appearant only with 270 kg/ha and 810 kg/ha. However, their maximum concentration was between 3.9 and 9.6 mg/kg.

The increased concentration of some elements such as Se, Mo, Sr, Cd and Cr in plant leaves as a result of high load in the soils poses serious problems for human and animal nutrition, therefore, this must be taken into consideration when leafy vegetables or fodder plants are to be cultivated intoxic element- and heavy metal- polluted soils.

The different plants show different response to the level of heavy and toxic metals compared to untreated control sample as shown in Table 4. As, Cd and Se were phytotoxic for spinach plant as they decreased significantly the amount of harvestable yield. The other elements were not phytotoxic even at highest load in soil. It was remarkable that Cr-treatment, which has been found phytotoxic for various plants, increased the yield from 21.6 to 27.9 g/100 plant indicating high adaptation of spinach plant to the high concentration of such element (Kádár, 1992). The most detrimental metal was Se which caused the plants to die out at dose of 270 and 810 kg/ha. The other harmful metal was Cd. Its phytotoxicity appeared on spinach even at the lowest dose (30 kg/ha).

4.4.2. Element treatments and pigment composition of spinach

Tables 5-7 show the effect of four doses of different elements on the pigment and tocopherol content of spinach leaves. As being the main factory of bioenergy in green tissues, especially leaves, chloroplasts are expected to

be affected by element stress in soil-plant system via activation or inhibition of many enzymes working inside and around their envelopes. In plant leaves chlorophylls and accompanying carotenoids are synthesized by light-dependent pathways. In addition, isomerization reactions of photosynthetic pigments can be regulated by certain metal-dependent enzymes. Through such mechanisms stress of an element can affect the composition and content of photosynthetic pigments (fat-soluble chlorophylls and carotenoids).

As shown in Table 5 the lutein and β-carotene content of the leaves increased with the addition of Cr till 270 kg/ha and declined when the dose was raised to 810 kg/ha. The highest level of carotenoids was found in Se-treated samples. This element when added to the soil at a dose of 30 and 90 kg/ha increased β-carotene content by 6.6 and 10.3 times, respectively, as related to the control (untreated). Zn- and Pb-treatments were detrimental to the biosynthesis of β-carotene. Such treatments caused β-carotene content to be under the estimated value in the control. The other elements induced favorable changes in the formation of β-carotene in spinach leaves when applied at a dose of 90 or 270 kg/ha, but at the highest dose there was marked impairment on β-carotene content. The aforementioned observations held true for lutein which showed similar tendency to that found with β-carotene as a function of element load in soil.

As regards chlorophyll a, the major pigment of spinach leaves, Cr-treatments caused gradual increase in its concentration and the concentration of its 10-epimer (chlorophyll a') with no destruction observed

even at the highest dose (Table 6). The effect of Cr-treatment on chlorophyll b was very interesting. The concentration of chlorophyll b tended to decrease proportionally to the increase of the element dose. With an inverse correlation chlorophyll b' increased (Figures 18 and 19) indicating that epimerization reaction was activated by Cr-stress in soil-plant system.

Like Cr, Mo affected the carotenoid content and chlorophyll composition of spinach leaves, in particular, its influence on epimerization of chlorophyll b to b'. With other group of elements such as Sr, Pb, Cu and Se pigment content was amended by the lowest dose and the epimerization reaction was induced only at the higher doses. These elements caused great degradation to the carotenoid profile of the leaf extract.

On the other hand, Hg-treatments showed no effect on epimerization of chlorophyll b nor a but the high load in soil caused marked degradation of all pigments. The only element that showed contrary manner on chlorophyll composition was Zn that activated conversion of 10-epimer to its original form ''chlorophyll b''.

It is worthy to mention that isomerization of carotenoid that have been reported by Biacs et al (1995) in carrot root was not observed in spinach leaves cultivated under the same agricultural conditions. This is due to the fact that in carrot carotenogenesis is a light-dependent process producing only carotenoids while in spinach leaves carotenoids are formed as a function of light-dependent pathway in which chlorophylls are the dominant photosynthetic pigments with high biological activity.

Data on Table 7 shows change in tocopherol content of spinach leaves as influenced by element treatments. Se-treatment exhibited detrimental effect on tocopherol content even at the beneficial concentration (the lowest doses). This reveals that Se interacts extremely with the biosynthesis of such an antioxidant. The other detrimental elements are Cr and As that caused significant decrease in the concentration of tocopherol at the highest doses (270 and 810 kg/ha).

The other elements had no significant effect on tocopherol, moreover, with the highest load of Hg in soil a slightly significant increase was recorded on tocopherol content of the leaves.

Regarding response of ascorbic acid (vitamin C) content to the element treatments it can be said that because of the high mobility of this acid as a metal carrier between plant parts there was a great variation between replicate samples and in many cases it was below the detection limit. To have reasonable results the whole plant would has been taken and homogenized for preparation of representative samples.

4.5 Model of in-vitro interaction between antioxidants and pigments

The concentration of endogenous antioxidants originating from food samples was too small. α-Tocopherol content was 2.2, 10.0 and 5.0 μg/ml of the model in parsley, tomato and spice red pepper extracts, respectively. These amounts can be neglected when compared to 200 μg/ml as the minimum concentration of the added antioxidant in the model system. In the

case of ascorbic acid, the extracts were free of this antioxidant, because of its solubility in water, it was removed with the water phase during extraction procedure.

The amounts of extracted lipids from parsley, tomato and ground paprika in the model system were found to be 8, 25 and 50 mg, respectively. These values represent lipid classes and fat-soluble pigments. Percentage of added antioxidants as related to the total amount of lipid was found to be: Ascorbic acid in parsley extract model was 10-68%, in tomato was 3.5-21% and in spice red pepper was 2-11%, while, tocopherol in parsley was 45-900%, in tomato was 14-250% and in spice red pepper was 10-120%.

4.5.1 Aqueous system (LOX-catalyzed pigment cooxidation)

The ability of LOX enzyme to catalyze reaction of pigment decolorization by cooxidation has long been known, and used both for qualitative assays of LOX activity and in commercial applications (Sanz et al. 1994).

The newly elaborated aqueous model opens new possibilities for more accurate determination of pigment degradation and susceptibility to oxidation than the spectrophotometric methods in which only one pigment can be examined. The HPLC separation of pigment individuals before and after incubation with LOX gives more data on sensitivity of each constituent in the system and its interaction with the others showing at the same time the

behavior of each pigment toward free radical chain reaction. Table 8 shows the data of 3-4 measurements of pigment retention (%) in the model after an incubation with LOX. The values for coefficient of variation (C.V.%) ranged among 5.3 and 9.9. The highest values were recorded with chlorophyll a from parsley, lycopene from tomato and CME from spice red pepper powder. The reason lies in the high sensitivity of those pigments to singlet oxygen and light during extraction and pre-injection preparation of the samples. Despite the fact that carrying out of four replications practically needs, at least, two days to finish the obtained C.V%. Values are regarded low taking into consideration the changing conditions from day to day, for instance, room temperature, activity of enzyme preparation, etc. In some cases when the replications were carried out on the same day, very small values could be obtained for C.V% (less than 3) indicating that this method is enough accurate and reliable.

4.5.1.1 Cooxidation of parsley-extract

Extract of parsley represent a mixture of naturally occurring chlorophylls and carotenoids. It is therefore, of interest to study degradation rate of the individuals of such pigment through LOX-catalyzed oxidation of unsaturated fatty acids. The different pigments showed different susceptibility to cooxidative degradation. In agreement with that reported by Daood and Biacs, (1989) the polar pigments such as lutein, chlorophyll b and chlorophyll a were very sensitive to LOX-catalyzed cooxidation with

chlorophyll a being the most degradable component, but it does not agree with Sanz et al. (1994) who found that LOX (extracted from chickpea) has no effect at all on chlorophylls. This may be explained by the fact that LOX cooxidation activity can vary both in terms of position and chirality; this variation is a function of the nature of the substrate, the reaction conditions, the source of enzyme and the type of isoenzyme used (Grosch et al. 1976, Ramadoss et al. 1978, Reynolds et al. 1982 and Zamora et al. 1988).

Although carotenoids are very sensitive to LOX-catalyzed cooxidation (Hildebrand et al. 1982 and Parry et al. 1991), β-carotene underwent oxidative degradation as fast as chlorophyll did. This result agrees with that reported by Sanz et al. (1994).

Effect of added ascorbic acid

Table 9 shows that addition of ascorbic acid improved stability of chlorophyll b and chlorophyll a toward cooxidative degradation. When added to the reaction mixture up to 0.2 mg/ml ascorbic acid raised the retention of chlorophylls from 38% to 90% after 30 minutes incubation with the enzyme. Further addition brought about no significant effect. As illustrated in Table 22 efficiency of ascorbic acid to prevent chlorophyll a is 1.35 times higher than that found with chlorophyll b. Furthermore, the highest [$\varepsilon^{0.1\,mg/ml}$] value with both chlorophyll b and a was estimated at the lowest concentration (0.05 mg/ml) of antioxidant, and gradually decreased as the concentration increased to 0.3 mg/ml.

Effect of addition of ascorbic acid on the stability of carotenoids (lutein and β-carotene) was similar to that found on chlorophylls (Table 9). With both chlorophylls and carotenoids, effect of addition of ascorbic acid on their retention followed first-order kinetic. However, [$\varepsilon^{0.1mg/ml}$] tended to decrease as the concentration increased as shown in Table 22.

It is to be mentioned that the maximum retention with added ascorbic acid was between 86% and 90%. Despite the high efficiency of ascorbic acid, as antioxidant, in the aqueous media complete retention of pigment could not be attained even at high concentration of the antioxidant, most likely due to the destruction of pigment by other factors such as light.

Effect of added tocopherol

Because of its insolubility in water, tocopherol was applied in the form of α-tocopherol acetate in the aqueous model system.

Table 10 shows the effect of α-tocopherol acetate on the oxidation of chlorophyll a, chlorophyll b, lutein and β-carotene. A slight protection to chlorophyll a was achieved by the addition of 0.2 and 1.1 mg/ml of α-tocopherol acetate. The maximum protection was obtained by the addition of 2.2 mg. Addition of 3.3 mg α-tocopherol acetate showed lower protection, most likely due to the decreased antioxidative efficiency of α-tocopherol as a function of increased concentration as reported by many authors (Cillard et al. 1980, 1986 and Terao et al. 1986). Furthermore, it has been reported

(Bazin et al. 1984, Jung et al. 1990, Fang et al. 1993 and Statue et al. 1995) that tocopherol may act as prooxidant when used at high concentration. This could be supported by the decreasing tendency of [$\varepsilon^{0.1mg/ml}$] of tocopherol at concentration over 2.2 mg/ml particularly with chlorophyll a where Δ retention was also decreased at high concentration of tocopherol (Table 23).

Concerning the effect of α-tocopherol acetate on the oxidation of chlorophyll b, more than 60% of chlorophyll b was oxidized when tocopherol was not added. Because of the low antioxidative activity of tocopherol in aqueous media as previously mentioned, only 50% of the oxidative damage of chlorophyll b could be reduced. At too high concentration (more than 2.2 mg) α-tocopherol acetate is scarcely effective.

Oxidation of lutein was almost as high as the chlorophylls. After 60 minutes about 70% of lutein was degraded. Addition of α-tocopherol acetate at different concentrations gave a slight protection to lutein with similar tendency shown on chlorophyll b.

The high degree of unsaturation in β-carotene structure renders it extremely susceptible to oxidation (Goldman et al. 1983). As illustrated in Table 10 about 60% of β-carotene was oxidized after 60 minutes of LOX-catalyzed cooxidation reaction.

Reduction of oxidative destruction of β-carotene by α-tocopherol acetate was recorded at relatively high concentration of the antioxidant (over 1.4 mg/ml) with scarce effect at high concentrations particularly at

60 minutes of the reaction time. It can be concluded that tocopherol acetate as a water soluble form of tocopherol exhibits less antioxidative activity in the aqueous media as compared to ascorbic acid. The pigment cooxidation could be reduced gradually as the amount of the of α-tocopherol increased (0.2-2.8 mg/ml reaction mixture). As compared to ascorbic acid, high amounts of α-tocopherol acetate was added. This is because of the low antioxidative activity of tocopherol in aqueous dispersions (Niki et al. 1986) which may be ascribed to lower mobility of α-tocopherol in aggregated medium (Gotoh et al. 1992) and/or hydrogen bonding by water at phenolic and/or chroman ether groups of α-tocopherol (Pryor et al. 1988, Barclay et al. 1989 and 1990). It can be noticed that the pigments were oxidized quickly at the beginning and then slowly at the end of cooxidation reaction in the control sample, while the added α-tocopherol samples were oxidized by different way, where the oxidation was almost quicker at the end than at the beginning, this may be due to the accumulation of oxidized tocopherol that acts as prooxidant in the media (Jung et al. 1992).

To study the interaction between antioxidant vitamins in their protection of fat-soluble pigments against LOX-catalyzed cooxidation, a mixture of ascorbic acid and tocopherol was added to the model system. The results indicated that synergism between the two antioxidants is evident after 60 minutes of the reaction when the antioxidative capacity of the mixture was higher than that of the individual antioxidant separately. This held true for both carotenoids and chlorophylls (Figures 20, and 21). These results agree

with that reported with many authors who have described experiments for elucidating interactions between vitamin C and vitamin E. Packer et al. (1979) showed that a hydrogen atom transfer reaction takes place between ascorbic acid and α-tocopheroxyl radicals. Bascetta et al. (1983) showed the quenching by vitamin C of α-tocopheroxyl radicals generated by oxidizing lipids on silica gel in an electron spin resonance (ESR) experiment. Barclay et al. (1983 and 1984) showed that there was a synergism between α-tocopherol and ascorbic acid using their respective chemically induced radical generating systems in an oil in water emulsion. On the other hand, our results do not agree with those reported by Tsuchihashi et al. (1995) who found that the combination of α-tocopherol or ascorbic acid with β-carotene did not exert marked positive effect. Ascorbic acid spared α-tocopherol but it did not spare β-carotene efficiently.

4.5.1.2 Cooxidation of tomato pigments

The LOX-catalyzed cooxidation of carotenoids extracted from tomato has also been studied. Extract of tomato products was selected in this work because of:

1. It contains only carotenoids of light-dependent carotenogenesis.
2. The carotenoids occur unesterified with fatty acids (like those of light-dependent in green vegetables).

3. Most of individual carotenoids of tomato-extract plays an important biological role in human and animal bodies.

Effect of added ascorbic acid

The effect of different ascorbic acid concentrations on the LOX-catalyzed cooxidation of lycopene, lycoxanthin and the major red pigments of tomato extract is illustrated in Table 11. With no ascorbic acid added (control) 52% and 63% of the original lycopene was lost in the system after 30 and 60 minutes of LOX-reaction respectively. Ascorbic acid, when added up to 0.2 mg/ml exhibited a good antioxidative activity and at 0.3 mg/ml it almost completely inhibited oxidative degradation of lycopene. The remarkable observation in this experiment was that at less effective concentrations at added ascorbic acid lycopene tended to decrease till 30 minutes of the reaction time and then gradually increased in the second half of the incubation time (60 minutes). This result gave a convincing evidence on the interaction between ascorbic acid and oxidized lycopene that led to partial regeneration of lycopene most likely due to the quenching by ascorbic acid of lycopene radicals.

Since lycoxanthin (hydroxy lycopene) showed similar behavior, it seemed that the interaction between ascorbic acid and lycopene derivatives is attributable to structural characteristic of the lycopene molecule. From data of Table 22, it could be concluded that effectiveness of ascorbic acid as a

function of increased concentration decreased with the increase of its level up to 0.2 mg/ml in the system, but at the maximum concentration there was a marked increase revealing that propagation and transfer of radicals between carotenoids and ascorbic acid molecules can de terminated only at high stress of ascorbic acid where its affinity to form stable complex is higher than its interaction with the other compounds.

β-Carotene showed different interaction with the added ascorbic acid. Since the value of both Δ retention and $[\varepsilon^{0.1mg/ml}]$ is in minus at the lowest ascorbic acid concentration (Table 22), it is evident that ascorbic acid behaved as a prooxidant and β-carotene is the most reactive free radical quencher under such conditions. On the other hand, such state could be modified (changed) by further supplementation of ascorbic acid that made Δ retention to increase and $[\varepsilon^{0.1mg/ml}]$ to be almost stable giving rise to a linear relation between Δ retention against cooxidation and concentration of added antioxidant.

β-Carotene was the most sensitive carotenoid to LOX-catalyzed cooxidation under the conditions used. This agrees with the results of Daood (1987) who used similar conditions of cooxidation reaction. Ascorbic acid when added even at high concentrations showed slight protective effect on β-carotene (Table 11) compared to its effect on lycopene and lycoxanthin. This may be explained by the big structural difference between β-carotene, lycopene and lycoxanthin. The presence of two phenyl rings on the molecule

of β-carotene seems to facilitate its rapid reaction with oxy radicals under certain conditions. Burton and Ingold (1989) and Seis (1991) reported that β-carotene has high reactivity as oxy radical and singlet oxygen quencher under low oxygen pressure.

Effect of added tocopherols

Although, the α-tocopherol concentration used was about 7 times higher than that of ascorbic acid, it could not protect the carotenoids against cooxidation as ascorbic acid did. Protection of red carotenoids (lycopene and lycoxanthin) by α-tocopherol acetate increased with increasing its concentration in the system (Table 12). The required concentration for tocopherol to be efficiently working in the aqueous system was 10 times more than that of ascorbic acid. Moreover only 70% and 80% of lycoxanthin and lycopene could be protected, respectively, against oxidation by adding 3.3 mg/ml of α-tocopherol acetate. It is of interest that the oxidation prevention coefficient [$\varepsilon^{0.1mg/ml}$] of tocopherol increased with the increase of antioxidant concentration in the system up to 1.1 mg/ml but tended to decrease with the further addition (Table 23). This held true with both lycopene and lycoxanthin with lycopene been protected more efficiently than lycoxanthin by α-tocopherol. The substantial reduction in the antioxidative efficiency of tocopherol as a function of increasing its concentration is most

likely due to the prooxidant effect of such antioxidant at high concentration as earlier described.

Unlike ascorbic acid α-tocopherol acetate had high antioxidative effect on β-carotene as illustrated in Table 12. This may indicated that β-carotene is less effective antioxidant in the presence of α-tocopherol in the lipid phase of the system. Linear relation is expected between Δ retention and α-tocopherol concentration up to 2.2 mg/ml, but further addition of α-tocopherol caused slight increase in Δ retention . The values of [$\varepsilon^{0.1mg/ml}$] which was constant in the concentration range between 0 and 2.2 mg/ml (Table 23) and decreased over this level supported the aforementioned expectation.

Effect of a mixture of 0.1 mg ascorbic acid and 1.1 mg α-tocopherol acetate on cooxidation of lycopene and β-carotene is shown in Figure 22. The synergestic effect of ascorbic acid on tocopherol which usually leads to a higher antioxidative effect as reported by some authors (Tappel et al. 1968, Packer et al. 1979, Niki et al. 1982, 1983 and 1984, and Lambelet et al. 1985) was noted with lycopene. The mixture of ascorbic acid (0.1mg) and α-tocopherol acetate (1.1 mg/ml) when applied gave higher protection of lycopene against LOX-catalyzed cooxidation than each antioxidant did separately. It is again evident that addition of ascorbic acid caused regeneration to the oxidized lycopene at the second period of the cooxidation reaction. In case of β-carotene (Figure 22) generally, β-carotene was highly

oxidized and it could not be protected by both ascorbic acid and α-tocopherol acetate revealing that the conditions are favorable for it to perform its antioxidant function.

4.5.1.3 Cooxidation of red paprika-extracted pigments

Paprika (Capsicum annuum) is one of the oldest and most important food colors. Its products are the sources of natural carotenoid concentrates. The effect of antioxidants on red or yellow pigment content of spice red pepper was studied against LOX-catalyzed cooxidation. Spice red pepper pigment contains some antioxidant carotenoids such as β-carotene which is present at high concentration. Presence of such antioxidants in this model system makes it necessary to elongate the incubation period from 60 to 90 minutes to be enough for studying the LOX-catalyzed cooxidation of the pigments. Because of high diversity of spice red pepper extract (with isocratic separation it contain about 40 peaks), we focused only on the major groups such as capsanthin monoesters (CME), β-carotene and capsanthin diesters (CDE).

Effect of added ascorbic acid

Capsanthin is the pigment that presents in high amount in the red pepper products. When the fruit fully ripens, it occurs esterified in the form of mono- and di-esters containing different fatty acids (Biacs et al. 1989 and

Minguez et al. 1994). Table 13 illustrates the effect of different concentrations of ascorbic acid on prevention of CME, CDE and β-carotene against cooxidative degradation. CMEs were extremely oxidized by LOX with 80% loss in the control experiment where no ascorbic acid added. Addition of ascorbic acid improved stability of CME fraction against cooxidation. Ascending tendency was observed for [$\varepsilon^{0.1mg/ml}$] in the range between 0.1 and 0.3 mg/ml with CME (Table 22) giving higher applicability of ascorbic acid to protect such pigment at higher concentrations with no prooxidant effect noticed. As concerns the relationship between Δ retention and antioxidant concentration, the data implied that the antioxidant was slightly effective at low concentration (0.05 mg/ml) followed by marked and almost linear increase in CME cooxidation prevention as the concentration increased. Since CDE fractions contain two fatty acids on the molecule and being chemically more stable toward oxidative degradation (Biacs et al. 1989), it is expected to have different interaction with the added antioxidants. At low level, ascorbic acid protected slightly CDEs, but at 0.2 mg/ml, the antioxidant was of high reactivity and at 0.3 mg/ml about 95% of CDEs were protected particularly in the first half of incubation period as. Unlike CMEs, CDEs exhibited low stability after 90 minutes of the reaction time. The effectiveness of ascorbic acid in CDE oxidation prevention was affected positively by the increased concentration of the antioxidant (Table 22). This was clear from the increase of Δ retention as a result of increased amount of added ascorbic acid. As regards the antioxidant

efficiency of ascorbic acid with CDE as a function of concentration, the [$\varepsilon^{0.1mg/ml}$] value was maximum with 0.2 mg/ml antioxidant and then decreased.

As for β-Carotene it was unstable against oxidation. About 70% of its content was lost as a result of LOX-catalyzed cooxidation (Table 13). With supplementation of ascorbic acid (0.3 mg/ml) 85% of the original content was retained by the system . The relation between added ascorbic acid and β-carotene degradation in red pepper extract is different from that earlier described in tomato extract. This may be due to the differences in the composition of these extracts. On contrast to what was found with CME and CDE, the tendency of change in [$\varepsilon^{0.1mg/ml}$] of ascorbic acid was descending with β-carotene revealing the less effectiveness of the antioxidant in protection of this type of carotenoid (Table 22). In addition, at the lower concentrations ascorbic acid protected β-carotene more efficiently than CME and CDE, but at higher concentration, the difference among the three groups in the their interaction with ascorbic acid became smaller.

Effect of added tocopherols

Table 14 illustrates the effect of α-tocopherol on the oxidation of CME, CDE and β-carotene. Protection of CME increases with the increasing α-tocopherol content. There was no prooxidative effect even at high concentration of α-tocopherol. The highest value of OP was estimated with

α-tocopherol concentration of 1.1 mg/ml over which the value decreased. Similar results were obtained with CDEs, with this fraction being more stable toward oxidation destruction. Concentration of 2.8 mg α-tocopherol gave the highest protection to CDEs during the first half of the incubation period. It seemed that addition of more antioxidant was less useful in oxidation prevention process.

The effect of α-tocopherol on cooxidation of β-carotene was similar to that found with CMEs (Table 14) where the highest antioxidant concentration (3.3 mg/ml) gave no more protection to β-carotene with the lowest [$\varepsilon^{0.1mg/ml}$]. In general, there was a slightly descending tendency of [$\varepsilon^{0.1mg/ml}$]change as a function of increased concentration with an equilibrium state over 2.2 mg/ml of added tocopherol, This seemed true for all carotenoid groups tested (Table 23). The finding that β-carotene can be protected by α-tocopherol in the first 30 minutes of reaction time and slowly destructed in the second 30 minutes supported those reported by Daood and co-worker (1985) who found that in red pepper extract the presence of ascorbic acid or tocopherol at considerably high level makes β-carotene acts as a second oxidation barrier.

Addition of ascorbic acid to α-tocopherol to enhance synergistic effect between them resulted in no significant variation except slight increase in the protection of β-carotene as a function of synergism between ascorbic acid and tocopherol (Figures 23 and 24) after 90 minutes of cooxidation reaction.

4.5.2 Non-aqueous system (photooxidation)

The effect of light on pigments' stability has been reported briefly in the literature (Cole et al. 1957 and Lovric et al. 1970). Due to light-permeable packaging or additional light-exposure situations pigment-containing products could lose important constituents. Therefore, more information on the effects of light on pigment-containing products is needed. Also, it is important to study the effect of antioxidants on their stability against light-catalyzed photooxidation. In this model, 2 hours incubation time was used in order to cause high level of photooxidative degradation of fat-soluble photosynthetic pigments.

Table 15 shows data on determination, by HPLC, of pigment retention by the non-aqueous model system (photooxidation). When 4 replications were carried out for the same samples of parsley, tomato and spice red pepper powder, the value of % C.V ranged between 4.6 and 10.6. It is to be mentioned that this range reveals the accuracy and reliability of this method and suitability of the model system for investigation of oxidative degradation of pigments

4.5.2.1 Photooxidation of parsley-extracted pigments

In green vegetables, chloropylls have been reported as sensitizers for singlet oxygen formation (Rawls et al. 1970 and Endo et al. 1984) and carotenoids as singlet oxygen quenchers (Foote et al. 1968 and 1970). This

can make complicated the interaction between antioxidants and photosynthetic pigment in the food model systems.

Generally, carotenoids are susceptible to isomerize in the presence of chlorophyll derivatives. It has been reported (Jensen et al. 1982, Ashikawa et al. 1986 and O'Neil et al. 1995) that a variety of chlorophyll derivatives such as chlorophyll a and b, pheophytin a and b and zinc pheophytin a and b complexes, have been found to act as effective photosensitizers inducing the formation of β-carotene cis isomers. The mechanism is believed to act via an excited state triplet of chlorophyll and its derivatives, as shown in the following proposed mechanism:

chlorophyll a + light $\rightarrow$ **^{1}chlorophyll a***

^{1}chlorophyll a* $\rightarrow$ **^{3}chlorophyll a***

^{3}chlorophyll a* + **β-carotene** $\rightarrow$ **chlorophyll a** + **^{3}β-carotene***

^{3}β-carotene* $\rightarrow$ **cis-β-carotene**

This state was not confirmed according to the results obtained in this work. The isomerization of all trans to cis form was observed on violaxanthin and lutein but not on β-carotene (Figure 25). It is believed that the results in the literature have been derived from in-vitro experiments based on testing only β-carotene in chlorophyll-sensitized photooxidation. So, in case of mixture of different carotenoids more polar xanthophylls such as violaxanthin and

lutein are more sensitive than β-carotene to photosensitization by chlorophylls. These results implied that under certain conditions polar xanthophylls may play more important role than that of β-carotene in the metabolism and transfer of light energy through plant tissues.

Effect of added ascorbic acid

Ascorbic acid was added to the model as ascorbyl palmitate because of the insolubility of ascorbic acid in carbon tetra-chloride which was used as free radical initiator solvent. This form is widely used as food ingredient to reduce oxidation of fat and fat-soluble materials. Table 16 illustrates degradation of chlorophyll a, chlorophyll b, lutein and β-carotene as a function of exposure to light and the effect of ascorbyl palmitate on their stability against photooxidation. Chlorophyll a which is three times more than chlorophyll b in plant leaves (Lichtenthaler et al. 1987), was more sensitive to photooxidation deterioration. The model system lost 35% and 70% after 60 and 120 minutes exposure to light, respectively. Ascorbic acid when added to the system showed good antioxidative effect which increased proportionally to the increased concentration.

Efficiency as a function of concentration [$\varepsilon^{0.1mg/ml}$]of ascorbyl palmitate in prevention of oxidative degradation of photosynthetic pigments in non-aqueous system was lower (>50 times) than that of ascorbic acid in the aqueous system. Moreover, addition of this material up to 1.1 mg/ml brought

about either slight or non significant oxidation prevention with all pigments tested. Data on Table 24 demonstrate that both Δ retention and [$\varepsilon^{0.1mg/ml}$]of the antioxidant were modified by increasing the concentration up to 3.3 mg/ml.

In case of chlorophyll b, it showed better stability toward photooxidative destruction as compared to chlorophyll a. This chlorophyll derivative was substantially protected by addition of 1.1 mg/ml of ascorbyl palmitate particularly after 60 minutes of photo-reaction. From data of Table 24 the value of Δ retention increased with increasing the concentration of the antioxidant, however, [$\varepsilon^{0.1mg/ml}$] was in descending tendency when the concentration was increased over 1.1 mg/ml. It seemed that chlorophyll b was actively interacting with oxidized ascorbyl palmitate over 1.1 mg/ml giving an evidence for its being the first oxidation barrier under the conditions used in non-aqueous model system.

As a function of reaction time both lutein and β-carotene was linearly decreasing through photooxidation course. This antioxidative activity of such carotenoid could be retarded only by addition of relatively high amounts of ascorbyl palmitate. 80% of β-carotene was photooxidized within 120 minutes, likely due to its action as antioxidant.

From data on Table 24, it is clear that rising the level of added ascorbyl palmitate up to 2.2 mg/ml resulted in no significant variation in its capacity to prevent carotenoid photooxidation as concluded from Δ retention

and [$\varepsilon^{0.1mg/ml}$] values. At higher concentration (3.3 mg/ml) there was remarkable protection of both carotenoid components by such an antioxidant with an improvement in the antioxidant efficiency, in term of Δ retention and [$\varepsilon^{0.1mg/ml}$], as a function of increased concentration.

Effect of added tocopherol

Iwatsuki et al. (1994) studied the effect of solvents and media on the antioxidant activity of α-tocopherol. He found that the antioxidant activity of α-tocopherol is smaller in protic solvents than in aprotic solvents. Accordingly, tocopherol acetate should exhibit higher antioxidation capacity in CCl_4 than in aqueous media.

As shown in Table 17 chlorophyll a had weaker response to α-tocopherol than to ascorbyl palmitate especially at the first 60 minutes of exposure to light. However, the rate of oxidative degradation was substantially decreased at the highest concentration used (3.3 mg/ml) in particular, after 60 minutes of the photooxidation reaction. Unlikely, chlorophyll b showed better response to the antioxidation by tocopherol. Rising the concentration of antioxidant to 3.3 mg/ml resulted in a high retention (about 95%) in the first period of photooxidation reaction.

As compared to ascorbyl palmitate α-tocopherol was found much more effective in prevention of pigment oxidation in non-aqueous system. This was concluded from the high values of Δ retention obtained with all pigments

tested. The values of Δ retention and [$\varepsilon^{0.1mg/ml}$] obtained at different concentration of added tocopherol gave convincing evidence on the linearity of relation between % retention and antioxidant concentration up to 3.3 mg/ml. Further improvement of oxidation prevention efficiency in terms of Δ retention and [$\varepsilon^{0.1mg/ml}$] with chlorophyll a could also be achieved (Table 25). In case of chlorophyll b similar tendency was observed except that at the highest concentration (4.4 mg/ml) there was no more increase in Δ retention and decrease of [$\varepsilon^{0.1mg/ml}$].

β-Carotene needed higher concentration of α-tocopherol to be protected than that needed for lutein. Since β-carotene does undergo trans-cis isomerization as mentioned earlier, its high sensitivity to photooxidation is attributable to oxygen-dependent degradation and light-induced destruction rather than chlorophyll sensitized reaction. This confirmed that during photooxidation, β-carotene is more susceptible than lutein in the presence of the sensitizer (chlorophyll a) and more antioxidant is practically required for the retardation of photosentisization reactions that make β-carotene highly sensitive to oxidative degradation. Although Δ retention was increasing proportionally to the increased concentration of antioxidant, [$\varepsilon^{0.1mg/ml}$] tended to slightly decrease with lutein (Table 25). In case of β-carotene, [$\varepsilon^{0.1mg/ml}$] approached maximum value at 2.2 mg/ml and then decreased slightly.

Shown in Figure 26 the effect of the antioxidants mixture on the photooxidation of chlorophyll a, where, the antioxidative effect of the

mixture was higher than the effect of ascorbyl palmitate and lower than that of α-tocopherol. No synergistic effect was observed when the two antioxidants were added to the system. This almost holds true for chlorophyll b oxidation as shown in Figure 26. The oxidation prevention was due rather to tocopherol.

The effect of the antioxidant mixture on photooxidation of β-carotene is shown in Figure 27. While ascorbyl palmitate showed no antioxidative effect to β-carotene at the applied concentration (1.1 mg/ml), the mixture showed almost the same protection to β-carotene as α-tocopherol acetate did. So, in this model system the uses of a mixture of antioxidants seem to be unuseful to improve antioxidative properties of the individual antioxidants against cooxidation of fat-soluble pigments.

4.5.2.2 Photooxidation of tomato-extracted pigments

Effect of added ascorbic acid

Ascorbic acid was used in a form of ascorbyl palmitate because of its insolubility in non-aqueous media. Table 18 shows the antioxidative effect of ascorbyl palmitate on photooxidation of lycopene, lycoxanthin and β-carotene. When added at low concentration ascorbyl palmitate showed prooxidative effect on these pigments. This agrees with the results obtained by some authors (Frankel et al. 1989 and Belitz et al. 1987). The protection of pigments was slightly improved as the antioxidant concentration increased

indicating the low antioxidation efficiency of such material in the non-aqueous system.

Function of ascorbyl palmitate in protection of lycopene was affected by increasing its concentration. Δ retention was increasing proportionally to the increase concentration with maximum [$\varepsilon^{0.1mg/ml}$] being obtained at 2.2 mg/ml (Table 24). As for the effect of concentration on prevention of lycoxanthin oxidation, similar tendency of change was observed on Δ retention, but the maximum value of [$\varepsilon^{0.1mg/ml}$] was estimated at the lowest concentration of the antioxidant (1.1 mg/ml) followed by marked decline as the concentration increased. It is worthy to mention that efficiency of ascorbyl palmitate to protect lycoxanthin was several times higher than that shown to protect lycopene. This reflects the high interaction between lycopene and oxidized form of ascorbyl palmitate.

The interaction between ascorbyl palmitate and β-carotene during photooxidation was different from that found with red carotenoids. In the first interval of exposure to light, addition of 0.2 mg/ml gave no antioxidative effect on β-carotene destruction. At higher concentration of the antioxidant, higher stability was observed on β-carotene during the first 60 minutes followed by marked decline in its retention by the system, which could be due the photooxidation of the other carotenoids that act as antioxidant such as lycopene (singlet oxygen quencher) as reported by Di Mascto et al. (1989) and Sies et al. (1991). The maximum Δ retention of

ascorbyl palmitate was recorded with concentration of 2.2 mg/ml over which prooxidant effect was evident as shown in Table 24. Since maximum value of [$\varepsilon^{0.1mg/ml}$] was achieved at the lowest concentration of added ascorbyl palmitate and descending tendency as the concentration increased, it can be said that at relatively high level of such an antioxidant β-carotene performs its antioxidative role, whereas at low levels it behaves as the second oxidation barrier where lycopene was acting as first oxidation barrier.

The interaction between ascorbyl palmitate and β-carotene from two extracts (parsley and tomato) was absolutely different. In presence of chlorophyll in the extract of parsley, it did not show any antioxidative properties while it was effective antioxidant in the extract of tomato where only carotenoids are existing.

Generally, photo-degradation data indicated that with no antioxidant added lycopene, the major carotenoid responsible for tomato color, degraded by similar way to that of provitamin A carotenoid, β-carotene. This manner is contrary to that found in the aqueous system as mentioned previously. The structure of lycopene differs slightly from those of β-carotene in the conjugated double bond system and it does not have ring structure at the ends of the molecule. These structural differences may account for differences in degradation rates (Pesek et al. 1987).

Effect of added tocopherols

The effectiveness of the tocopherol on photooxidation of red carotenoids from tomato differs from that of ascorbyl palmitate. Up to 3.3 mg/ml it showed high protection to red carotenoids, while at higher concentration (4.4 mg/ml) α-tocopherol exhibited lower antioxidant potency, most probably due to the prooxidant effect of the excessive amount of the antioxidant (Table 19). As reported by Jung et al. (1992), Loury et al. (1966), Bazin et al. (1984) and Fang et al. (1993) oxidized tocopherol can act as prooxidant at high concentration. However, addition of tocopherol disturbed partially further decomposition of both lycoxanthin and lycopene at the second half time of photooxidation reaction (120 minutes). According to these results it was demonstrated that lycoxanthin is much easier to be protected by tocopherol than lycopene which shows better antioxidant activity in non-aqueous system.

The estimated values of Δ retention showed an equilibrium state reached between 0 and 3.3 mg/ml of the antioxidant followed by dramatic decrease that gives strong evidence on the prooxidant effect of the antioxidant at high concentration. This was supported by the descending tendency of $[\varepsilon^{0.1\,mg/ml}]$ especially with 4.4 mg/ml α-tocopherol. The same held true for lycoxanthin oxidation prevention function of tocopherol which decreased extremely at the highest applied concentration (Table 25).

Approximately 60% of β-carotene was photooxidized during 120 minutes in the control sample where no antioxidant was added as shown in

Table 18. High antioxidative effect of tocopherol was observed when different antioxidant concentrations were added. More than 90% of β-carotene was protected when 2.2 mg/ml war added. Like with lycopene and lycoxanthin, addition of tocopherol up to 4.4 mg/ml gave lower protection of β-carotene.

The effect of concentration of tocopherol on its efficiency in terms of Δ retention and [$\varepsilon^{0.1mg/ml}$] is shown in Table 25. The increased concentration led to marked decrease in the Δ retention as well as [$\varepsilon^{0.1mg/ml}$] revealing the prooxidant effect of tocopherol and antioxidant role of β-carotene. Basing on the estimated values of Δ retention and [$\varepsilon^{0.1mg/ml}$] concentration of 1.1 mg/ml tocopherol is optimum for the best antioxidant effect of this antioxidant under the conditions of photooxidation model system. It is also interesting that tocopherol protected β-carotene more efficiently than lycopene. This revealed that β-carotene has lower antioxidant activity than lycopene in tomato extract. This may explain why lycopene has high biological activity as bioantioxidant in human or animal body (Sies et al. 1991).

The effect of the mixture on the main tomato carotenoids (lycopene and β-carotene) is shown in Figure 28. The mixture of the antioxidants showed no higher antioxidative effect on photooxidation of lycopene than that obtained from adding 1.1 mg/ml ascorbyl palmitate, giving convincing evidence on the absence of synergism between ascorbyl palmitate and tocopherol in protection of lycopene against photooxidative damage.

The antioxidative effect of the mixture on photooxidation of β-carotene was similar to that found on lycopene except that presence of tocopherol beside ascorbyl palmitate neutralized the prooxidant effect of the later giving rise to better stability of β-carotene. These results are of practical importance particularly when β-carotene must be stored in food or synthetic preparation for a long time with high biological activity as provitamin A.

4.5.2.3 Photooxidation of red pepper-extracted pigments

Due to presence of some carotenoids in spice red pepper which act as antioxidants, the photooxidation rate is slower than that of parsley and tomato, so that, the exposure to light time in this model was extended to 180 minutes to induce more photooxidative degradation of the different carotenoids of spice red pepper extract.

Effect of added ascorbic acid

The antioxidative effect of ascorbyl palmitate on photooxidation of CME, CDE and β-carotene is illustrated in Table 20. After addition of 0.3 mg/ml ascorbyl palmitate photo-degradation of CME fraction occurred with higher rate than that observed with the control indicating the high sensitivity of CMEs to the high reactive free radicals produced as a result of light-catalyzed oxidation of the antioxidant. It should be noted that increasing the concentration of ascorbyl palmitate beyond 1.1 mg/ml reduced oxidative destruction of CME all the time of exposure to light.

The maximum Δ retention was achieved with the application of 3.3 mg/ml of ascorbyl palmitate. It should be said that the concentration of ascorbyl palmitate would be more than 3.3 mg/ml to approach complete protection of CME against photooxidative damage (Table 24). The values in minus of Δ retention and [$\varepsilon^{0.1mg/ml}$] indicates convincingly the prooxidant effect of ascorbyl palmitate on CME fraction which seems to interact very rapidly with the reactive oxygen species on ascorbyl palmitate produced by photooxidation. This prooxidant effect could be reduced or neutralize by excessive addition of the antioxidant that resulted in an increasing of Δ retention and [$\varepsilon^{0.1mg/ml}$].

In case of CDEs, prooxidative effect of the antioxidant was observed only with addition of low antioxidant concentration (0.3 mg/ml) and after 180 minutes of exposure to light. In general, CDE fraction was more stable than CME toward photooxidation, likely due to the presence of two fatty acids (mainly saturated) on the molecule of capsanthin. This agrees with the results appeared in literature (Minguez-Mosquera et al. 1995 and Biacs et al. 1989). The data on Table 24 concerning Δ retention and [$\varepsilon^{0.1mg/ml}$] support the aforementioned observations.

As shown in Table 18, of studied pigments β-carotene was the most sensitive to photooxidative degradation. Exposure to light of 180 minutes caused β-carotene to lose 70% of its initial content. This also reflects the antioxidative action of β-carotene. This result agrees with that reported by

Minguez-Mosquera et al. (1995) and Pesek et al. (1987). At lower concentration (up to 1.1 mg/ml) there was prooxidant effect of ascorbyl palmitate on β-carotene during the first 2 hours. Such an effect was neutralize by further supplementation of the antioxidant. The antioxidant function of ascorbyl palmitate was performed efficiently, in particular during the first period of exposure to light (90 minutes), at concentration of 3.3 mg/ml. The decline of the curve of retention % versus time after 90 minutes confirmed the antioxidant trend of β-carotene when the whole system is exposed to high oxidation stress. Addition of antioxidant up to 3.3 mg/ml increased retention of β-carotene to 87% and 59% after 90 and 180 minutes of exposure to light respectively. As compared to the control, these values are considerably high.

The effect of concentration of ascorbyl palmitate on its efficiency in prevention of β-carotene oxidation was similar to that found on its efficiency in prevention of CME oxidation with β-carotene being more susceptible than CME to prooxidant effect of ascorbyl palmitate particularly at low concentrations (Table 24).

Effect of added tocopherols

The antioxidative effect of α-tocopherol on photooxidation of CME, CDE and β-carotene is shown in Table 21. There was no prooxidant effect of tocopherol as exhibited by ascorbyl palmitate. The best antioxidation

prevention of tocopherol could be noted when added at 1.6 mg/ml and excessive addition was scarcely effective. As shown in Table 25, increasing the concentration of tocopherol to 1.1 and 2.2 mg/ml caused Δ retention to increase to 2.5 and 11, and [$\varepsilon^{0.1mg/ml}$] to 0.2 and 0.5, respectively.

Similar antioxidative effect of tocopherol on photooxidation of CDEs was observed as shown in Table 21. After the addition of 2.2 mg/ml tocopherol, photooxidative degradation occurred very slowly within 3 hours and reached an equilibrium state. Concerning the effect of concentration of tocopherol on its oxidation prevention, the maximum Δ retention and [$\varepsilon^{0.1mg/ml}$] were obtained when 2.2 mg/ml of tocopherol was applied (Table 25).

β-Carotene shows the same behavior of CDE fraction except that after 180 minutes of exposure to light, even the lowest level of added tocopherol, was effective in its protection against photooxidative damage. By addition of 2.2 mg/ml tocopherol, retention of β-carotene could be increased 150% (from 30% to 80%) while the same added amount improved retention of CME and CDE by 66% and 57% respectively. Thus, basing on those facts β-carotene is playing a role of second oxidation barrier when the effective antioxidants are present at relatively high concentration and first oxidation barrier when the antioxidation capacity of the system is very low as in the control samples (no added antioxidant). From data on Table 25, it was evident that doubling the concentration of tocopherol would result in

manifolding the value of Δ retention and having the same value of [$\varepsilon^{0.1mg/ml}$], that means high linearity in the relation between concentration and % retention of β-carotene.

The effect of antioxidant mixture of 0.5 mg/ml tocopherol and 2.2 mg/ml ascorbyl palmitate on protection of the major carotenoid fractions of spice red pepper from photooxidation is shown in Figure 29 and 30. With exposure to light of 90 minutes no significant effect of such a mixture on the protection of any of the tested fractions. Slight synergistic effect between the antioxidants was manifested in the increase of pigment retention by the model system after exposure to light of 180 minutes. Basing on these results, it is recommended to use mixture of such antioxidant for better storage stability of natural carotenoid preparations produced from spice red pepper such as oleoresins that may be exposed to light during processing and long-term storage.

5. SUMMARY

Summary of new scientific results

1. Ascorbic acid was found the most effective endogenous antioxidant during ripening of spice red pepper and tomato protecting fat-soluble pigments and tocopherols from oxidative degradation. Its dynamical changes depended on its initial concentration at early stages of ripening.
2. The antioxidant function of tocopherols and carotenoids decreased substantially when the food system lost considerable quantities of the available water (overripened or dried).
3. Both α-tocopherol and ascorbic acid provided first oxidation barrier while β-carotene provided second oxidation barrier against oxidative degradation of pigments during a 120-day storage of ground red pepper.
4. Soil pollution with some heavy and toxic metals was found to activate, to high extent, isomerization reactions of photosynthetic pigments leading to substantial compositional changes. Among the applied elements Cr, Mo, Pb, Cu, Se and Sr promoted conversion of chlorophyll to its 10-epimer, whereas Hg, Zn, and Cd activated the reverse reaction.
5. It was interesting that despite its high phytotoxicity Se improved pigment content and enhanced better antioxidant effect of tocopherol in spinach leaves. Similar effect was observed for Mo and Cr, but at the highest applied doses. The other elements had no significant effect on tocopherol content.

6. In the aqueous model antioxidant activity of ascorbic acid was much higher than that of tocopherol acetate. The effective concentration of ascorbic acid was 10 times lower than that needed for α-tocopherol acetate to provide the same protection of photosynthetic pigments against LOX-catalyzed cooxidation. Ascorbic acid provided high oxidation prevention coefficient [$\varepsilon^{0.1mg/ml}$], particularly at low concentrations. The highest Δretention and [$\varepsilon^{0.1mg/ml}$] values were recorded with chlorophyll a in parsley extract, lycopene in tomato extract and β-carotene in red pepper extract. β-Carotene performed marked antioxidant function in tomato extract with the lowest concentration of added ascorbic acid.
7. At low concentration ascorbic acid had remarkable interaction with lycopene and lycoxanthin from tomato extract. This interaction led to a partial regeneration of these carotenoids after 60 minutes of reaction time under the conditions of the model system.
8. Different figure of antioxidant-pigment interaction was obtained when α-tocopherol acetate was added to the aqueous model system. The best prevention was found with chlorophyll b from parsley, β-carotene from tomato and CME from red pepper. No antioxidant effect noticed for β-carotene at all the applied concentrations of α-tocopherol acetate.
9. In non-aqueous photooxidation model, chlorophyll-sensitized trans to cis isomerization of carotenoids was repeatedly noticed on polar xanthophylls, but not on β-carotene, of parsley extract. Such a light-

catalysed reaction could be influenced by addition of effective antioxidants.

10. Antioxidant activity of ascorbyl palmitate in the non-aqueous model was approx. Fifty times less than that of ascorbic acid in the aqueous system. With chlorophyll b from parsley extract, lycoxanthin and β-carotene from tomato and CDE from red pepper [$\varepsilon^{0.1mg/ml}$] of ascorbyl palmitate was decreasing as the concentration increased from 1.1 to 3.3 mg/ml. With other pigments from other extracts the value of [$\varepsilon^{0.1mg/ml}$] was either stable or decreasing as a function of increased concentration of antioxidant.
11. At low concentration of added ascorbyl palmitate both CMEs and β-carotene from red pepper extract had remarkable antioxidant activity. This was evident from the negative values of Δretention and [$\varepsilon^{0.1mg/ml}$] of ascorbyl palmitate obtained with these pigments.
12. As compared to ascorbyl palmitate, α-tocopherol provided better prevention against photooxidative damage of carotenoids. About 95% of the pigments was retained by the system when α-tocopherol was added at concentration of 1.6 - 4.4 mg/ml. With lutein from parsley and all examined carotenoids from tomato [$\varepsilon^{0.1mg/ml}$] of α-tocopherol tended to decreased as the concentration increased, while with other pigments it was either stable or increasing.

13. Under the conditions of aqueous model system ascorbic acid when added together with tocopherol showed slight synergistic effect that led to a better protection of pigments toward LOX-catalyzed cooxidation. This held true with all pigments tested except β-carotene. In water free model no synergism was noticed between the two antioxidants.

Applicability of the results

- The results are highly applicable in the fields of food technology, postharvest technology, pharmaceutical industry and cosmetics.
- Determination of the effective concentration of natural antioxidants would assist in achieving the best quality and the longest shelf-life of several foods, medicinal, and cosmetic products.
- From the economical point of view it is also of interest that combination of bioantioxidants in a suitable ratio can reduce the costs of production of any product.
- In the processing of plant oil-derived products the obtained results are useful to determine which form of antioxidant vitamins and at which concentration can be applied, with no prooxidant effect, to minimize oxidative deterioration.

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8. TABLES

Table 1 : Applied treatments of element load in soil, 1991 calcareous loamy chernozem, Nagyhörcsök, Hungary.

Element signal	Treatment kg/ha				Applied forms
	0/30*	90	270	810	
*As**	30	90	270	810	$As_2O_3/NaAsO_2$(4:4.3)
*Cd**	30	90	270	810	$CdSO_4 \cdot 8/3H_2O$
Cr	-	90	270	810	K_2CrO_4
Cu	-	90	270	810	$CuSO_4 \cdot 5H_2O$
*Hg**	30	90	270	810	$HgCl_2$
Mo	-	90	270	810	$(NH_4)_6Mo_7O_{24}.4H_2O$
Pb	-	90	270	810	$Pb(NO_3)_2$
*Se**	30	90	270	810	Na_2SeO_3
Sr	-	90	270	810	$SrSO_4$
Zn	-	90	270	810	$ZnSO_4 \cdot 7H_2O$

Table 2: HPLC determination of antioxidant vitamins in fresh and dried spice red pepper samples. (Km-622 cultivars, 1994, Kalocsa)

Samples	Concentration of antioxidant vitamins	
	Fresh spice red pepper	Dried spice red pepper
	β-carotene μg/g DM	
1	419	610
2	407	624
3	434	631
4	447	650
5	468	652
X-	435	633
SD	24	18
C.V%	5.5	2.8
	α-tocopherol μg/g DM	
1	430	598
2	393	654
3	422	622
4	380	642
5	402	611
X-	405	625
SD	20.6	23
C.V%	5.1	3.7
	Ascorbic acid mg/g DM	
1	10.5	9.2
2	10.1	9.4
3	10.7	9.8
4	10.4	9.5
5	10.9	9.1
X-	10.5	9.4
SD	0.3	0.27
C.V%	2.8	2.9

C.V.% = Percent coefficient of variation

$LSD_{5\%}$= Least significant difference at 5% probablity level

Table 3 : Effect of toxic and heavy metaltreatments on the element content (mg/kg) of spinach leaves. Chernezom soil , Nagyhörcsök , 1996

Element	Dose Kg/ha , spring 1991					
	0/30*	**90**	**270**	**810**	**LSD $_{5\%}$**	**P**
As*	000	0.00	0.92	3.94	0.62	< 0.001
Cd*	71.20	81.70	106.5	144.5	8.2	< 0.05
Cr	1.90	2.89	7.25	16.25	0.6	< 0.001
Cu	-	7.96	10.80	18.50	2.6	< 0.05
Hg*	0.00	0.00	3.10	9.56	1.77	<0.01
Mo	82.0	223.0	411.5	670.5	89.7	< 0.05
Pb	0.93	0.59	1.32	1.79	0.9	NS
Se*	141.1	765.0	#	#	14..9	< 0.001
Sr	-	164.5	268.0	518.0	28.0	< 0.01
Zn	57.3	163.0	41.5	289.0	27.9	< 0.01

$LSD_{5\%}$ = Least significant difference at 5% probability level.
p = probability level
NS = not significant
\# = the plant died out

Table 4 : Effect of toxic and heavy metal treatments on the yield of spinach leaves (g/100 plant, dry weight based). Chernezom soil , Nagyhörcsök , 1996

Element	**Dose Kg/ha , spring 1991**				
	0/30*	**90**	**270**	**810**	**LSD $_{5\%}$**
*As**	23.2	16.6	13.2	7.5	
*Cd**	16.5	13.4	12.2	4.3	
Cr	21.6	22.1	25.5	27.9	
Cu	-	24.5	23.2	26.5	
*Hg**	20.5	26.7	26.8	31.3	**7.0 ***
Mo	17.2	19.5	21.6	24.3	
Pb	20.7	21.0	24.0	20.7	
*Se**	30.2	21.1	-	-	
Sr	-	23.8	28.2	21.5	
Zn	20.9	22.8	20.9	21.1	
$LSD_{5\%}$	**10.1***				

C.V.% (percent coefficient of variance) = 16.4 (for the whole treatments).
$LSD_{5\%}$ = Least significant difference at 5% probability level.
*** $p < 0.05$**
 # = The plant died out

Table 5 : Effect of toxic and heavy metaltreatments on the β-carotene and lutein content of spinach leaves (μg/g. f.wt). Chernezom soil , Nagyhörcsök , 1996

Element	Dose Kg/ha , spring 1991				
	0/30*	**90**	**270**	**810**	**LSD $_{5\%}$**
		β- carotene			
*As**	5.3	8.5	2.6	2.7	
*Cd**	4.1	8.0	1.5	1.9	
Cr	3.1	7.0	11.1	9.0	
Cu	3.1	14.0	7.5	4.7	
*Hg**	3.1	4.9	2.7	1.5	**0.9*** **
Mo	3.1	3.3	6.8	6.8	
Pb	3.1	2.0	3.0	2.3	
*Se**	19.7	31.4	#	#	
Sr	3.1	3.5	6.5	0.9	
Zn	3.1	1.2	1.2	1.3	
$LSD_{5\%}$		**1.2*** **			
		Lutein			
*As**	73.3	83.3	71.7	48.0	
*Cd**	79.0	102.5	76.0	76.6	
Cr	70.1	78.8	88.3	60.2	
Cu	70.1	91.3	75.3	84.0	
*Hg**	68.5	65.0	62.3	70.3	**7.6*** **
Mo	70.1	69.4	77.2	91.3	
Pb	70.1	65.7	82.7	81.1	
*Se**	198.5	166.0	#	#	
Sr	70.1	70.2	95.2	96.9	
Zn	70.1	67.5	67.7	69.1	
$LSD_{5\%}$		**7.3*** **			

C.V.% (percent coefficient of variance) = 10.4 for β-carotene and 5.0 for lutein (for the whole treatments)

$LSD_{5\%}$ = Least significant difference at 5% probability level.

***P < 0.001**

 # = The plants died out

Table 6a : Effect of toxic element and heavy metal treatments on the chlorophyll a and á content of spinach leaves (μg/g. f.wt). Chernezom soil, Nagyhörcsök, 1996.

Element	Dose Kg/ha , spring 1991				
	0/30*	**90**	**270**	**810**	**LSD $_{5\%}$**
			Chlorophyll a		
*As**	92.2	96.3	77.3	65.2	
*Cd**	88.5	95.2	84.1	80.1	
Cr	90.6	112.4	114.5	128.1	
Cu	90.6	130.0	116.9	117.1	
*Hg**	91.8	69.9	79.8	79.5	**11.3*** **
Mo	90.6	107.2	104.5	126.9	
Pb	90.6	95.2	106.9	121.0	
*Se**	118.3	154.3	*	*	
Sr	90.6	85.8	110.2	108.1	
Zn	90.6	78.4	72.0	80.1	
LSD$_{5\%}$		**13.1*** **			
			Chlorophyll a`		
*As**	5.9	9.3	5.4	4.9	
*Cd**	5.5	8.1	5.6	5.8	
Cr	5.5	11.7	12.3	11.7	
Cu	5.5	16.4	9.9	5.3	
*Hg**	5.8	3.5	3.7	1.7	**1.8*** **
Mo	5.5	6.7	6.3	9.8	
Pb	5.5	8.8	7.1	3.3	
*Se**	14.4	20.8	*	*	
Sr	5.5	6.2	13.1	11.0	
Zn	5.5	3.8	2.9	3.7	
LSD$_{5\%}$		**1.7*** **			

C.V.% (percent coefficient of variance) = 5.7 for chlorophyll a and 12.2 for chlorophyll á (for the whole treatments)

LSD$_{5\%}$ = Least significant difference at 5% probability level.

***P < 0.001**

 # = The plants died out

Table 6b: Effect of toxic element and heavy metal treatments on the chlorophyll b and b′ content of spinach leaves (μg/g. f.wt). Chernezom soil, Nagyhörcsök, 1996.

Element	Dose Kg/ha , spring 1991				
	0/30*	90	270	810	LSD 5%
			Chlorophyll b		
*As**	25.1	23.5	23.7	12.8	
*Cd**	21.2	32.5	22.7	18.0	
Cr	23.0	19.8	3.3	0.2	
Cu	23.0	28.4	4.4	2.4	
*Hg**	23.2	29.4	31.2	29.6	**4.1*** **
Mo	23.0	21.3	17.1	9.3	
Pb	23.0	29.2	34.3	3.1	
*Se**	45.4	54.6	#	#	
Sr	23.0	37.0	24.2	14.5	
Zn	23.0	23.4	34.3	36.3	
$LSD_{5\%}$		**4.2*** **			
			Chlorophyll b′		
*As**	2.6	1.4	2.0	0.0	
*Cd**	2.9	5.1	1.0	0.2	
Cr	2.7	6.4	25.7	23.9	
Cu	2.7	6.2	24.0	10.6	
*Hg**	1.6	0.0	0.0	0.0	**3.0*** **
Mo	2.7	7.9	14.5	21.1	
Pb	2.7	3.4	2.8	18.4	
*Se**	2.6	20.0	#	#	
Sr	2.7	0.0	12.3	4.8	
Zn	2.7	2.1	0.0	0.0	
$LSD_{5\%}$		**2.8*** **			

C.V.% (percent coefficient of variance) = 9.2 for chlorophyll b and 4.0 for chlorophyll b′ (for the whole treatments)

$LSD_{5\%}$ = Least significant difference at 5% probability level.

***P < 0.001**

 # = The plants died out

Table 7: Effect of toxic element and heavy metal treatments on the α-tocopherol content of spinach leaves (μg/g. f. wt.). Chernezom soil, Nagyhörcsök, 1996.

Element	Dose Kg/ha , spring 1991				
	0/30*	90	270	810	LSD 5%
*As**	24.0	25.5	22.8	13.5	
*Cd**	22.3	24.2	24.1	26.4	
Cr	24.8	25.2	19.7	14.3	
Cu	24.8	22.6	23.3	23.7	
*Hg**	23.7	26.8	25.5	27.3	**4.55*** **
Mo	24.8	29.5	32.1	18.1	
Pb	24.8	24.9	23.0	27.0	
*Se**	14.5	13.0	#	#	
Sr	24.8	23.8	22.7	22.9	
Zn	24.8	25.3	25.6	25.0	
$LSD_{5\%}$	**4.5*** **				

C.V.% (percent coefficient of variance) = 9.2 (for the whole treatments)
$LSD_{5\%}$ = Least significant difference at 5% probability level.
***P < 0.001**
 # = The plants died out

Table 8: Determination, by HPLC method, of LOX-catalysed co-oxidation of photosynthetic pigments from parsley, tomato and spice red pepper in aqueous model system at 60 min (for parsley and tomato extract) and 90 min (for red pepper extract)

Pigments	Replications				$\bar{x}$	SD	C.V.%
	1	2	3	4			
				Parsley			
Chlorophyll a	28.4	28.8	24.4	27.3	27.2	1.7	6.3
Chlorophyll b	32.7	36.4	34.0	30.1	33.3	2.3	7.0
β-carotene	38.6	34.2	39.5	42.0	38.6	2.8	7.3
				Tomato			
Lycopene	40.0	34.0	38.6	42.0	38.6	2.9	7.5
β-carotene	11.5	10.0	12.0	11.0	11.1	0.7	6.3
				Red pepper			
CME	18.7	18.1	20.9	23.1	20.2	2.0	9.9
CDE	45.8	43.8	42.5	37.8	42.5	2.9	6.9
β-carotene	27.9	30.0	29.1	26.1	28.3	1.5	5.3

$\bar{x}$ = mean

SD = standard deviation

C.V.% = % coefficient of variation

Table 9: Effect of added ascorbic acid on retention of persley-extracted pigments by the model system of lipoxygenase-catalyzed linoleic acid oxidation.

Pigments and reaction time	**Added ascorbic acid concentration (mg/ml)**				
	0	0.05	0.1	0.2	0.3
Chlorophyll a					
30 min	38.5	71.0	82.5	88.2	89.5
60 min	27.2	66.0	74.0	82.0	83.7
Chlorophyll b					
30 min	51.0	75.0	80.0	82.7	87.2
60 min	33.3	69.0	76.9	78.8	82.7
Lutein					
30 min	47.2	71.2	79.6	82.0	85.5
60 min	30.9	55.7	72.7	79.1	81.5
β-carotene					
30 min	51.2	72.3	80.9	87.5	89.5
60 min	38.6	63.0	73.5	86.0	85.0

The values represent average of two replications.

Table 10 : Effect of added tocopherol acetate on retention of persley-extracted pigments by the model system of lipoxygenase-catalyzed linoleic acid oxidation.

Pigments and reaction time	**Added tocopherol acetate concentration (mg/ml)**				
	0	0.2	1	2.2	3.3
Chlorophyll a					
30 min	38.5	41.4	44.4	81.0	74.5
60 min	27.2	29.7	31.7	55.0	53.4
Chlorophyll b					
30 min	51.0	56.3	60.7	78.6	80.0
60 min	33.3	35.1	36.4	51.1	48.0
Lutein					
30 min	47.2	52.3	60.0	74.7	80.0
60 min	30.9	32.0	34.9	52.0	50.0
β-carotene					
30 min	51.2	76.0	73.2	86.0	88.0
60 min	38.6	42.4	50.0	54.2	52.2

The values represent average of two replications

Table 11: Effect of added ascorbic acid on retention of tomato-extracted pigments by the model system of lipoxygenase-catalyzed linoleic acid oxidation.

Pigments and reaction time	**Added ascorbic acid concentration (mg/ml)**				
	0	0.05	0.1	0.2	0.3
Lycopene					
30 min	47.2	56.0.	50.0	62.6	92.4
60 min	38.6	53.0	65.0	70.0	93.3
Lycoxanthin					
30 min	35.4	36.3	51.0	52.6	79.4
60 min	29.0	34.0	52.0	53.7	78.8
β-carotene					
30 min	27.0	25.0	31.5	37.4	40.6
60 min	11.1	11.9	16.3	20.1	25.0

The values represent average of two replications

Table 12: Effect of added α-tocopherol acetate on retention of tomato-extracted pigments by the model system of lipoxygenase-catalyzed linoleic acid oxidation.

Pigments and reaction time	**Added tocopherol acetate concentration (mg/ml)**				
	0	0.6	1.1	2.2	3.3
Lycopene					
30 min	47.2	50.0	71.6	78.8	84.0
60 min	38.6	42.0	64.9	74.0	80.0
Lycoxanthin					
30 min	35.4	43.0	58.9	67.0	72.7
60 min	29.0	31.4	52.0	61.1	67.0
β-carotene					
30 min	27.0	38.0	54.0	75.0	77.0
60 min	11.1	14.0	42.0	64.0	69.0

The values represent average of two replications.

Table 13: Effect of added ascorbic acid on retention of red pepper-extracted pigments by the model system of lipoxygenase-catalyzed linoleic acid oxidation.

Pigments and reaction time	**Added ascorbic acid concentration (mg/ml)**				
	0	0.05	0.1	0.2	0.3
CME					
45 min	32.0	41.0	42.0	58.2	75.8
90 min	20.2	30.0	32.0	46.1	68.2
CDE					
45 min	54.1	58.4	61.0	87.7	94.0
90 min	42.5	47.0	47.0	66.0	76.3
β-carotene					
45 min	39.0	67.0	68.0	74.0	84.0
90 min	28.3	44.0	47.0	62.0	70.0

The values represent average of two replications.

Table 14 : Effect of added α-tocopherol acetate on retention of red pepper-extracted pigments by the model system of lipoxygenase-catalyzed linoleic acid oxidation .

Pigments and reaction time	**Added tocopherol acetate concentration (mg/ml)**			
	0	1.1	2.2	3.3
CME				
45 min	32.0	65.9	71.8	82.9
90 min	20.2	51.7	64.5	72.0
CDE				
45 min	54.1	68.0	75.2	84.0
90 min	42.5	62.8	67.0	80.0
β-carotene				
45 min	39.0	73.1	76.0	86.4
90 min	28.3	54.0	69.0	71.0

The values represent average of two replications.

Table 15: Determination, by HPLC method, of photooxidation of photosynthetic pigments from parsley, tomato and spice red pepper in aqueous model system at 120 min (for parsley and tomato extract) and 180 min (for red pepper extract)

Pigments	Replications				$\bar{x}$	SD	C.V.%
	1	2	3	4			
				Parsley			
Chlorophyll a	29.0	24.7	30.0	29.7	28.4	2.1	7.2
Chlorophyll b	45.0	42.3	39.0	38.4	41.2	2.7	6.5
β-carotene	22.0	21.3	24.0	23.2	22.6	1.0	4.6
				Tomato			
Lycopene	36.5	37.7	31.1	32.0	34.3	2.8	8.2
β-carotene	41.0	48.4	39.6	36.5	41.4	4.4	10.6
				Red pepper			
CME	58.1	57.0	50.2	47.3	53.2	4.5	9.6
CDE	48.7	60.0	54.0	53.0	53.9	4.0	7.0
β-carotene	34.2	30.0	32.3	28.3	31.2	2.2	7.2

$\bar{x}$ = mean

SD = standard deviation

C.V.% = % coefficient of variation

Table 16 : Effect of added ascorbyl palmitate on retention of persley-extracted pigments by the model system of photo-catalyzed oxidation of linolei acid.

Pigments and reaction time	**Added ascorbyl palmitate concentration (mg/ml)**				
	0	1.1	2.2	3.3	4.4
Chlorophyll a					
60 min	64.0	65.3	73.5	79.6	92.0
120 min	28.4	34.0	38.6	48.5	53.3
Chlorophyll b					
60 min	72.2	80.2	82.0	83.0	84.7
120 min	41.2	45.0	47.7	55.4	61.2
Lutein					
60 min	56.8	59.0	59.9	65.6	68.8
120 min	30.2	26.5	29.8	40.8	45.3
β-carotene					
60 min	54.7	56.1	55.8	64.8	69.6
120 min	22.6	13.1	19.2	34.7	39.6

The values represent average of two replications.

Table 17: Effect of added α-tocopherol on retention of persley-extracted pigments by the model system of photo-catalyzed oxidation linoleic acid.

Pigments and reaction time	**Added α-tocopherol concentration (mg/ml)**			
	0	1.1	2.2	3.3
Chlorophyll a				
60 min	64.0	66.0	68.0	73.6
120 min	28.4	31.0	38.0	58
Chlorophyll b				
60 min	71.2	77.0	87.0	92.0
120 min	41.2	48.0	70.0	81.0
Lutein				
60 min	65.8	66.7	70.0	75.5
120 min	30.2	60.0	63.0	73.6
β-carotene				
60 min	54.7	58.0	70.0	80.5
120 min	22.6	23.0	48.0	75.0

The values represent average of two replications.

Table 18 : Effect of added ascorbyl palmitate on retention of tomato-extracted pigments by the model system of photo-catalyzed linoleic acid oxidation.

Pigments and reaction time	**Added ascorbyl palmitate concentration (mg/ml)**				
	0	0.2	1.1	2.2	3.3
Lycopene					
60 min	66.0	50.2	67.6	70.5	71.4
120 min	34.3	24.9	37.8	40.3	40.6
Lycoxanthin					
60 min	59.0	53.0	66.7	70.0	71.3
120 min	38.0	24.7	44.0	52.0	50.5
β-carotene					
60 min	67.5	69.2	84.5	91.1	84.8
120 min	41.4	32.7	44.8	50.0	50.0

The values represent average of two replications.

Table 19 : Effect of added α-tocopherol on retention of tomato-extracted pigments by the model system of photo-catalyzed linoleic acid oxidation.

Pigments and reaction time	**Added tocopherol concentration (mg/ml)**				
	0	1.1	2.2	3.3	4.4
Lycopene					
60 min	66.0	82.1	83.6	83.2	68.0
120 min	34.3	68.5	75.0.	70.0	60.0
Lycoxanthin					
60 min	59.0	73.3	77.3	83.1	71.4
120 min	38.0	65.0	73.3	78.0	63.0
β-carotene					
60 min	67.5	96.0	95.5	91.0	89.0
120 min	41.4	91.0	93.0	87.0	80.2

The values represent average of two replications.

Table 20 : Effect of added ascorbyl palmitate on retention of red pepper-extracted pigments by the model system of photo-catalyzed linoleic acid oxidation.

Pigments and reaction time	**Added ascorbyl palmitate concentration (mg/ml)**				
	0	0.2	1.1	2.2	3.3
CME					
90 min	80.0	74.0	78.7	85.0	91.0
180 min	53.2	44.0	63.4	64.0	68.0
CDE					
90 min	74.0	72.0	86.0	88.0	91.3
180 min	53.9	45.0	72.0	68.0	78.0
β-carotene					
90 min	71.4	61.6	58.1	72.0	87.0
180 min	31.2	35.7	42.8	45.2	59.0

The values represent average of two replications.

Table 21 : Effect of added α-tocopherol on retention of red pepper-extracted pigments by the model system of photo-catalyzed linoleic acid oxidation.

Pigments and reaction time	**Added tocopherol concentration (mg/ml)**				
	0	0.5	1.1	1.6	2.2
CME					
90 min	80.0	80.5	83.0	90.0	91.0
180 min	53.2	56.0	60.0	82.0	83.0
CDE					
90 min	74.0	74.5	78.0	83.0	90.0
180 min	53.9	55.0	59.0	78.0	83.0
β-carotene					
90 min	71.4	72.2	78.5	84.0	85.7
180 min	31.2	43.0	69.5	73.0	80.3

The values represent average of two replications.

Table 22 : Oxidation prevention of ascorbic acid in LOX-catalyzed cooxidation of fat-soluble pigments from parsley, tomato and spice red pepper in the model system at the half time of oxidation reaction

Pigments	Concentration mg/ml							
	Δ retention (%)				$[\varepsilon^{0.1mg/ml}]$			
	0.05	0.1	0.2	0.3	0.05	0.1	0.2	0.3
Parsley								
chlorophyll a	32.5	44.0	49.6	51.0	65.0	44.0	2.5	17.0
chlorophyll b	24.0	29.0	31.7	36.3	48.0	29.0	15.9	12.1
lutein	24.0	32.4	34.8	40.0	48.0	32.4	17.4	11.3
β-carotene	21.1	29.7	36.3	38.1	42.2	29.7	18.2	12.7
Tomato								
lycopene	8.8	1.2	15.4	45.0	17.6	11.8	7.7	15.0
lycoxanthin	0.9	15.6	17.2	44.1	1.8	15.6	8.6	14.7
β-carotene	-1.9	4.5	10.4	13.5	-4.0	4.5	5.2	4.5
Paprika								
CME	9.0	10.0	26.2	43.8	18.0	10.0	13.1	14.6
CDE	4.3	7.0	33.6	40.0	8.6	6.9	16.8	13.3
β-carotene	28.0	29.0	19.8	45.0	56.0	29.0	9.9	15.0

Table 23 : Oxidation prevention of tocopherol in LOX-catalyzed cooxidation of fat-soluble pigments from parsely, tomato and spice red pepper in model system at the half time of oxidation reaction.

Pigments	Concentration mg/ml					
	Δ retention (%)			$[\varepsilon^{0.1mg/ml}]$		
	1.1	2.2	3.3	1.1	2.2	3.3
Parsley						
chlorophyll a	4.6	42.5	42.2	0.4	1.9	1.3
chlorophyll b	7.6	27.5	34.3	0.7	1.3	1.1
lutein	10.0	27.5	45.5	0.9	1.3	1.4
β-carotene	i7.3	37.6	43.2	1.6	1.9	1.3
Tomato						
lycopene	24.4	31.7	37.0	2.2	1.4	1.1
lycoxanthin	23.5	31.7	37.3	2.1	1.4	1.1
β-carotene	27.5	48.0	50.2	2.5	2.2	1.5
Paprika						
CME	33.9	39.8	50.8	3.1	1.8	1.6
CDE	13.9	21.1	30.0	1.3	1.0	0.9
β-carotene	34.1	37.0	47.5	3.1	1.7	1.5

Table 24 : Oxidation prevention of ascorbyl palmitate in photooxidation of fat-soluble pigments from parsley, tomato and spice red pepper in themodel system at the half time of oxidation reaction.

Pigments	Concentration mg/ml					
	Δ retention (%)			$[\varepsilon^{0.1mg/ml}]$		
	1.1	2.2	3.3	1.1	2.2	3.3
Parsley						
chlorophyll a	1.3	9.5	15.5	0.1	0.4	0.5
chlorophyll b	9.0	10.8	11.9	0.8	0.5	0.4
lutein	2.2	3.1	8.9	0.2	0.2	0.3
β-carotene	1.4	1.1	10.2	0.1	0.05	0.3
Tomato						
lycopene	1.0	4.4	5.3	0.1	0.2	0.2
lycoxanthin	7.7	11.0	12.2	0.7	0.5	0.4
β-carotene	17.1	23.5	17.2	1.6	1.1	0.5
Paprika						
CME	-1.3	5.1	10.9	-0.1	0.2	0.3
CDE	12.0	14.1	17.2	1.1	0.6	0.5
β-carotene	-13.3	0.7	15.5	-1.2	0.03	0.5

Table 25 : Oxidation prevention of α-tocopherol in photooxidation of fat-soluble pigments from parsely, tomato and spice red pepper in model system at the half time of oxidation reaction.

Pigments	Concentration mg/ml							
	Δ retention (%)				$[\varepsilon^{0.1mg/ml}]$			
	1.1	2.2	3.3	4.4	1.1	2.2	3.3	4.4
Parsley								
chlorophyll a	0.2	4.0	6.0	9.7	0.2	0.2	0.2	0.2
chlorophyll b	5.7	15.8	20.8	20.7	0.5	0.7	0.6	0.5
lutein	9.9	13.2	17.2	18.5	0.9	0.6	0.5	0.4
β-carotene	3.3	15.2	18.2	25.6	0.3	0.7	0.6	0.6
Tomato								
lycopene	16.1	17.6	17.2	2.2	1.5	0.8	0.5	0.05
lycoxanthin	14.3	18.3	24.1	12.3	1.3	0.9	0.7	0.3
β-carotene	28.6	28.0	23.4	21.6	2.6	1.3	0.7	0.5
Paprika								
CME	2.5	11.0	10.4	-	0.2	0.5	3.2	-
CDE	4.0	16.1	16.6	-	0.4	0.7	5.0	-
β-carotene	7.2	14.3	13.8	-	0.7	0.6	4.2	-

9. FIGURES

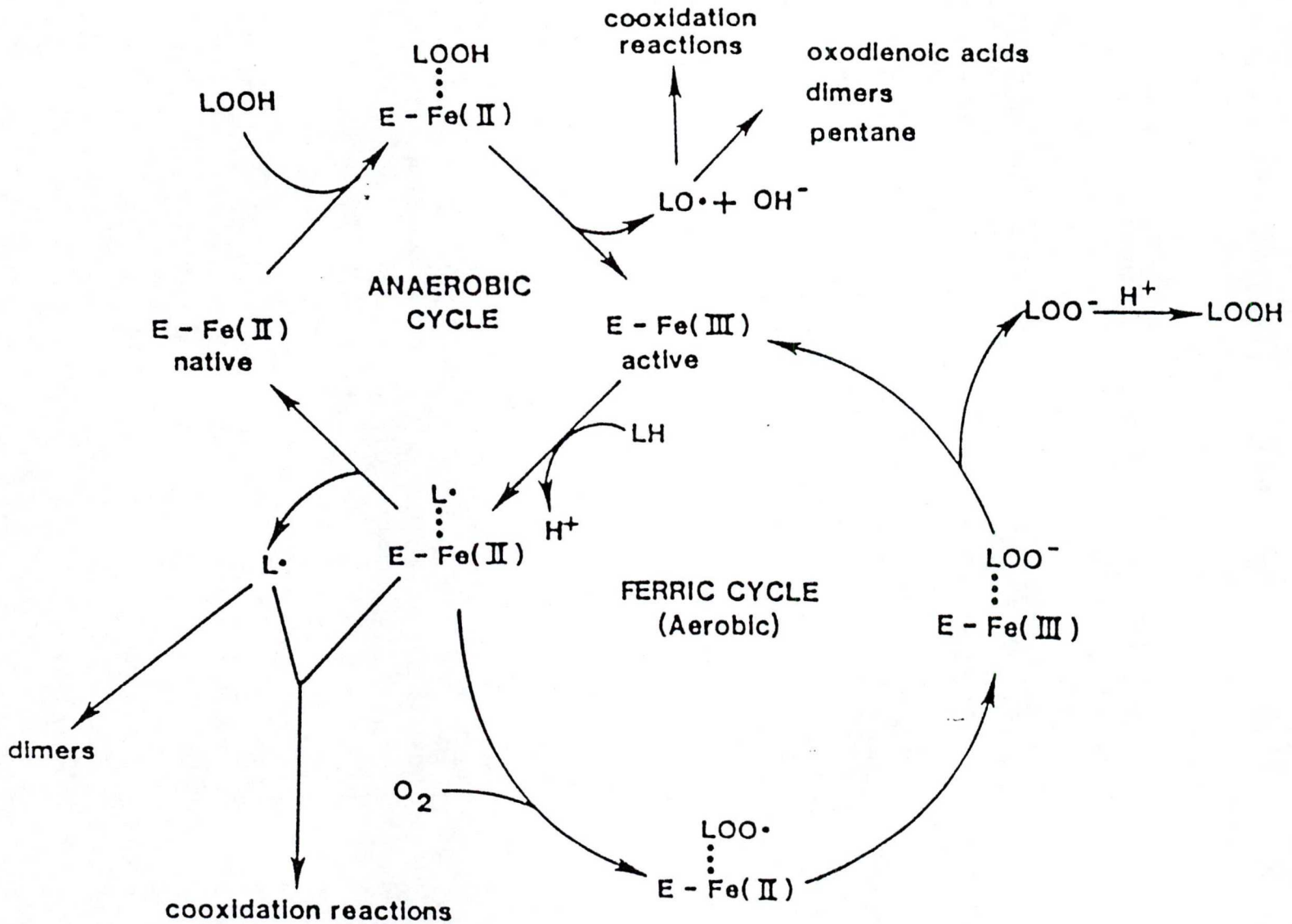
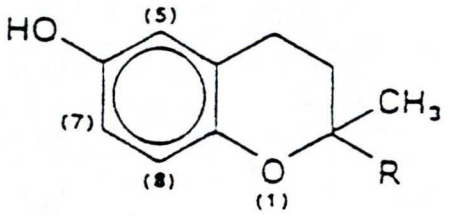
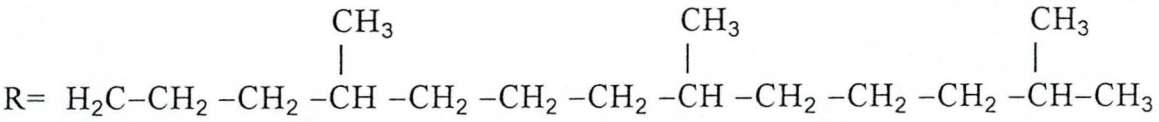


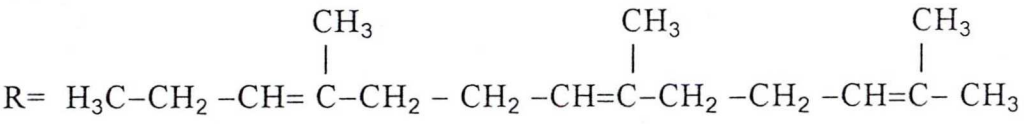
Figure 1 : Cooxidation of carotenoids (Robinson et al 1995)



Tocopherol:

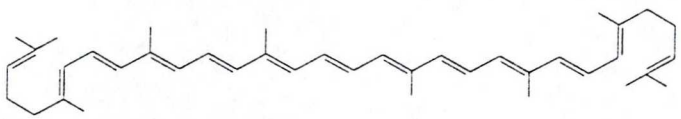


Tocotrienol:

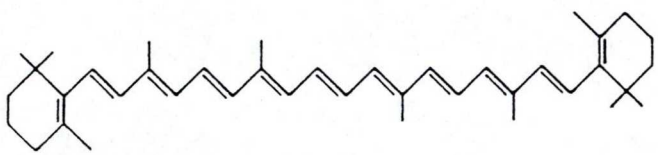


Substitution	*Tocopherols(T)*	*Tocotienols(T-3)*
5,7,8- Trimethyl	**α-T**	**α-T-3**
5,8- Dimethyl	**β-T**	**β-T-3**
7,8- Dimethyl	**γ-T**	**γ-T-3**
8- Methyl	**δ-T**	**δ-T-3**

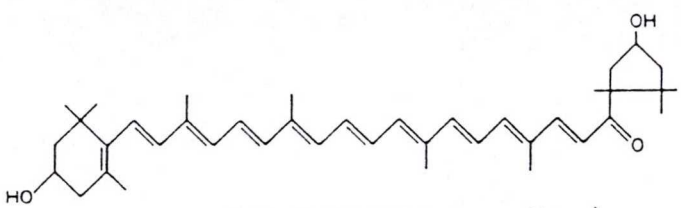
Figure 2: Tocopherols and Tocotrienols present in food.



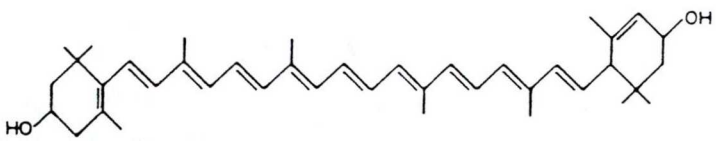
Lycopene (ψ,ψ-carotene)



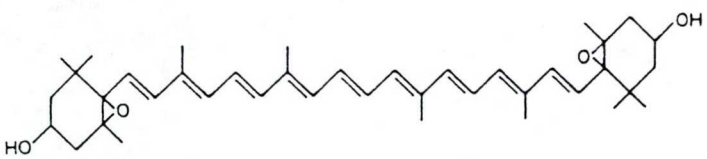
β-Carotene (β,β-carotene)



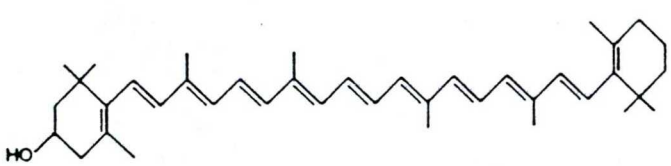
Capsanthin (3,3'-dihydroxy-β,κ-caroten-6'-one)



Lutein (β,ε-carotene-3,3'-diol)

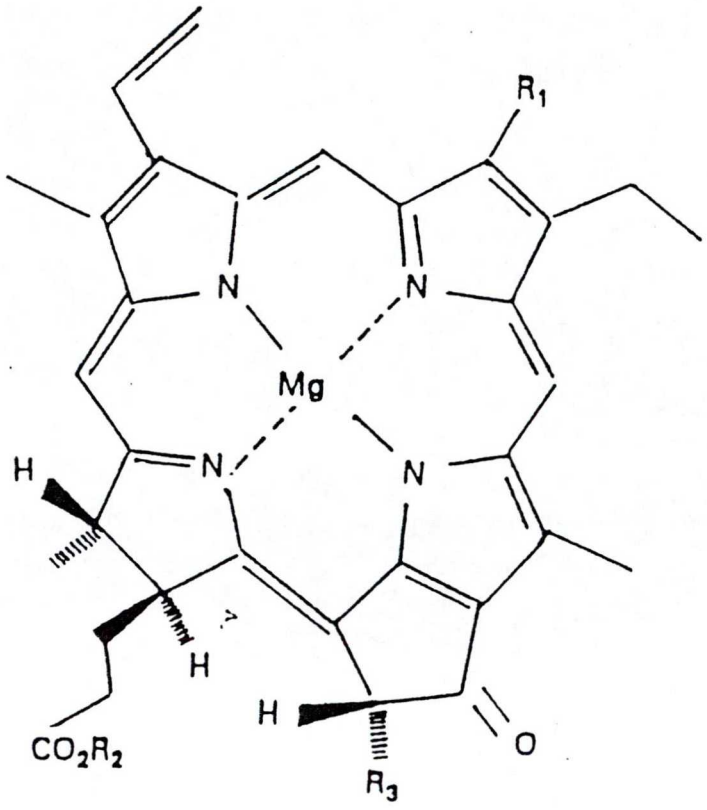


Violaxanthin (5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro-β,β-carotene-3,3'-diol)



β-Cryptoxanthin (β,β-caroten-3-ol)

Figure 4 : Structure formulas of some carotenoids



Compounds	Mg	R_1	R_2	R_3
Chlorophyll a	+	CH_3	Phytol	CO_2CH_3
Chlorophyll b	+	CHO	Phytol	CO_2CH_3
Chlorophyllide a	+	CH_3	H	CO_2CH_3
Chlorophyllide b	+	CHO	H	CO_2CH_3
Pheophytin a	–	CH_3	Phytol	CO_2CH_3
Pheophytin b	–	CHO	Phytol	CO_2CH_3
Pyropheophytin a	–	CH_3	Phytol	H
Pyropheophytin b	–	CHO	Phytol	H
Pheophorbide a	–	CH_3	H	CO_2CH_3
Pheophorbide b	–	CHO	H	CO_2CH_3

Figure 5 : Structural formulas of chlorophyll a and b and their derivatives.

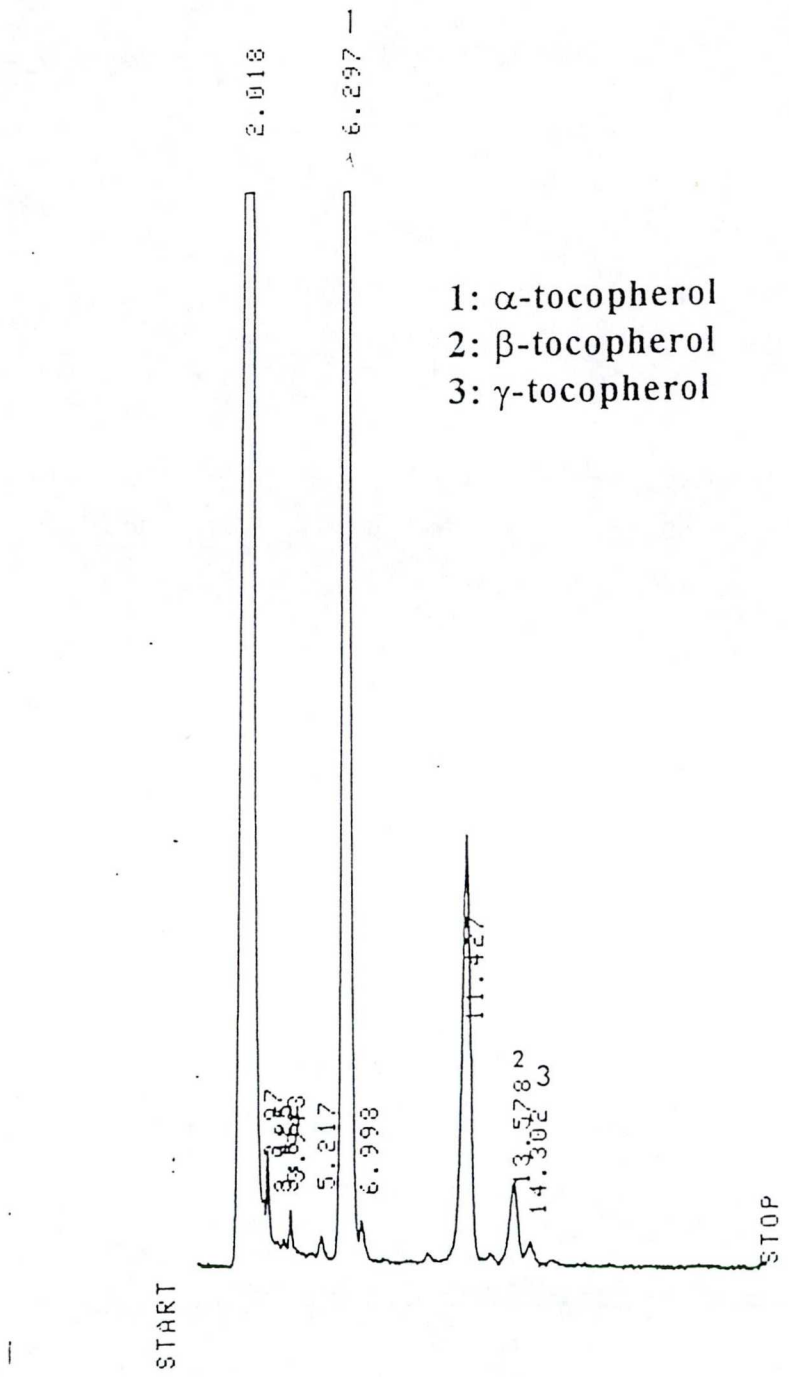


Figure 6 : HPLC separation of tocopherol analogues from spice red pepper (without seeds).

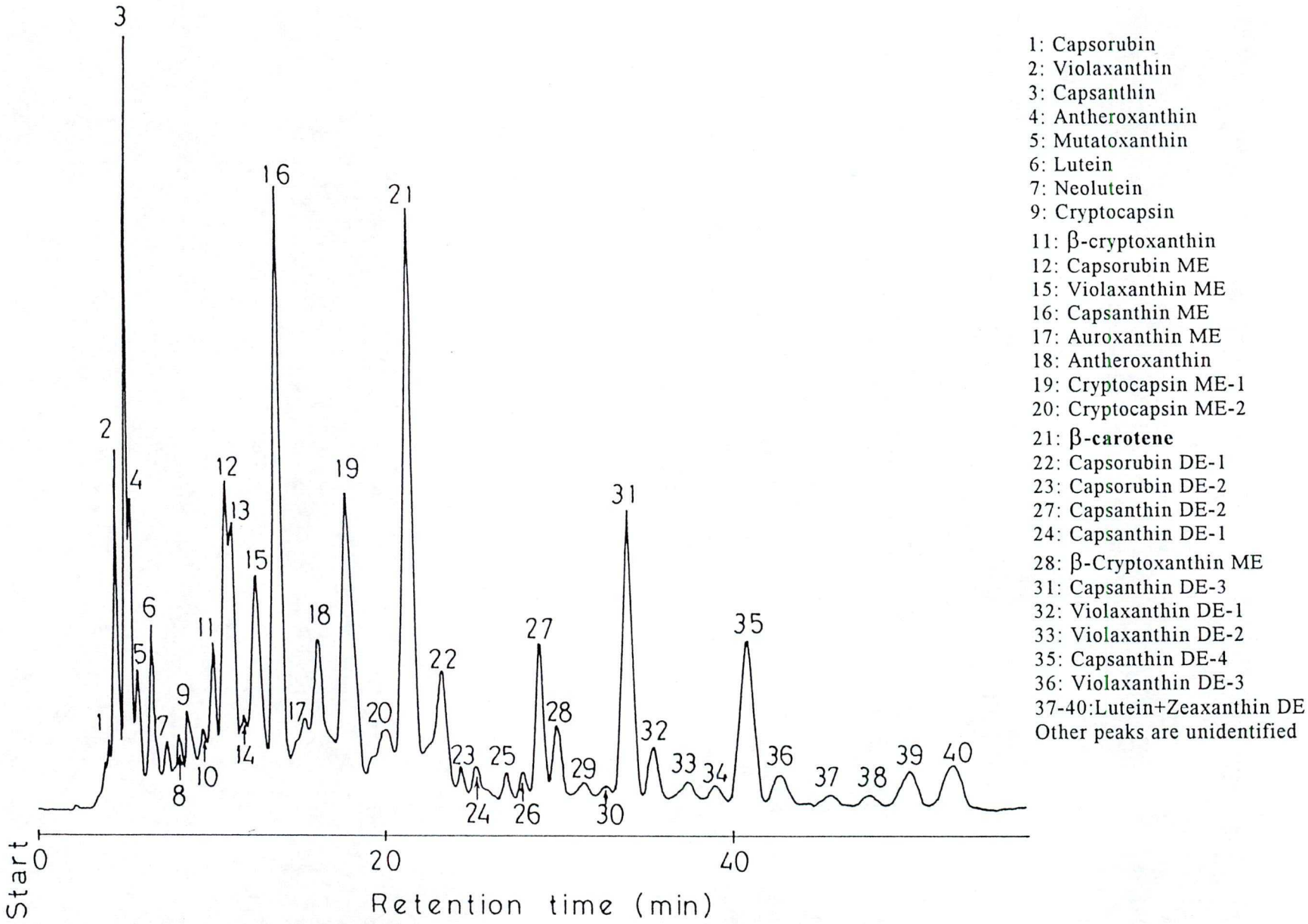


Figure 7 : HPLC separation of carotenoids and carotenoid esters from spice red pepper extract.

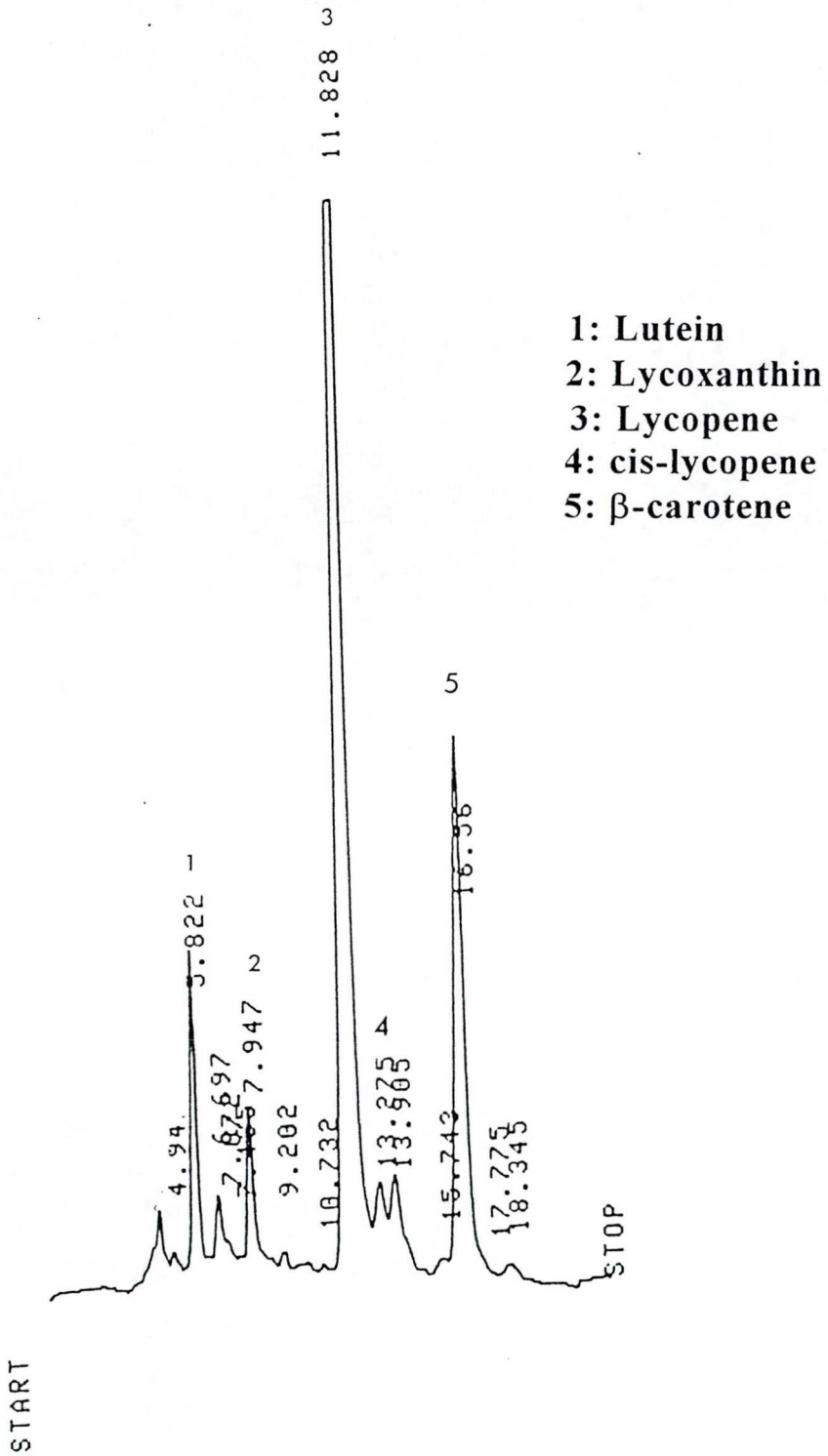


Figure 8 : HPLC separation of carotenoids from tomato.

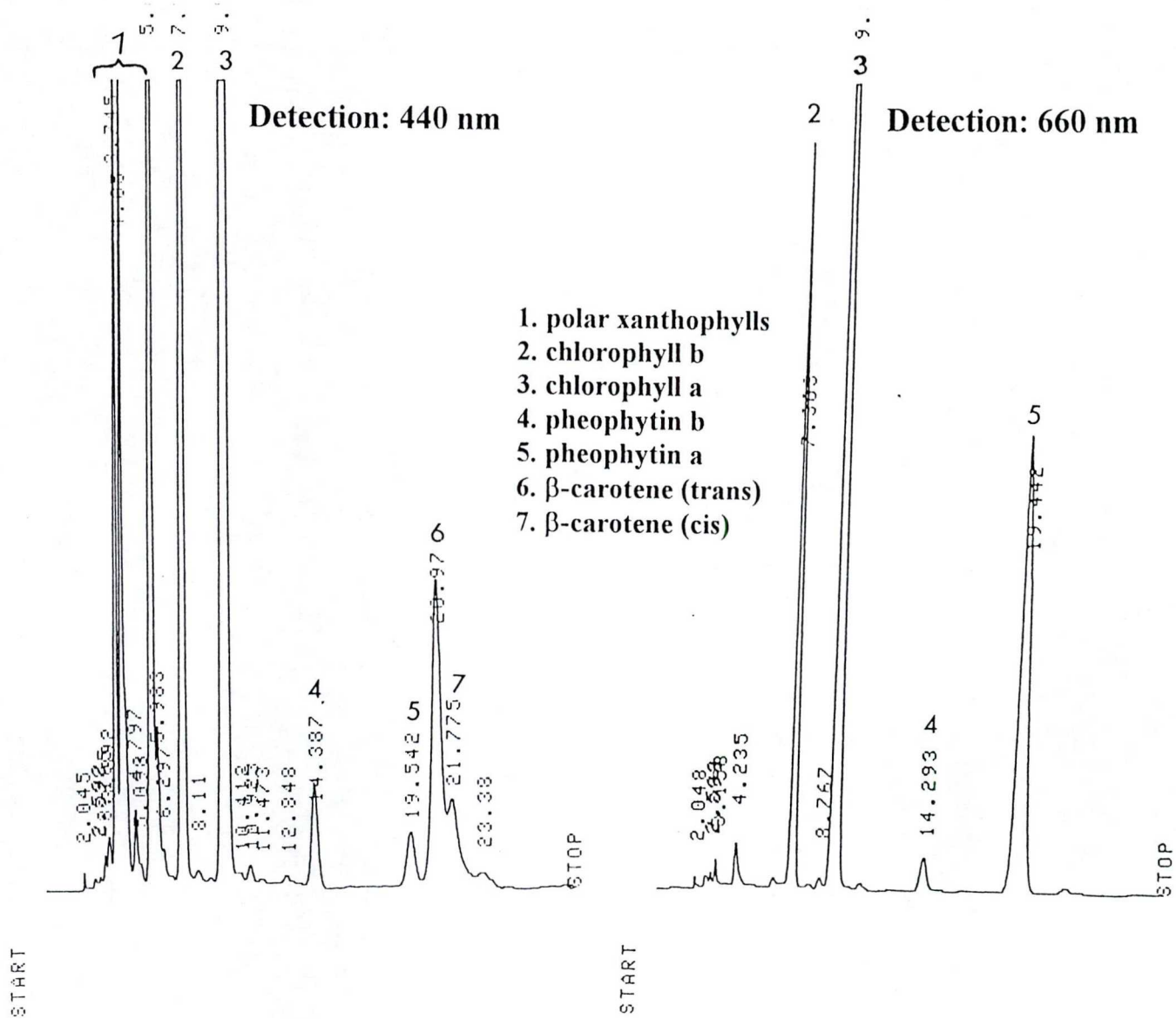


Figure 9 : HPLC separation of carotenoids from parsley.

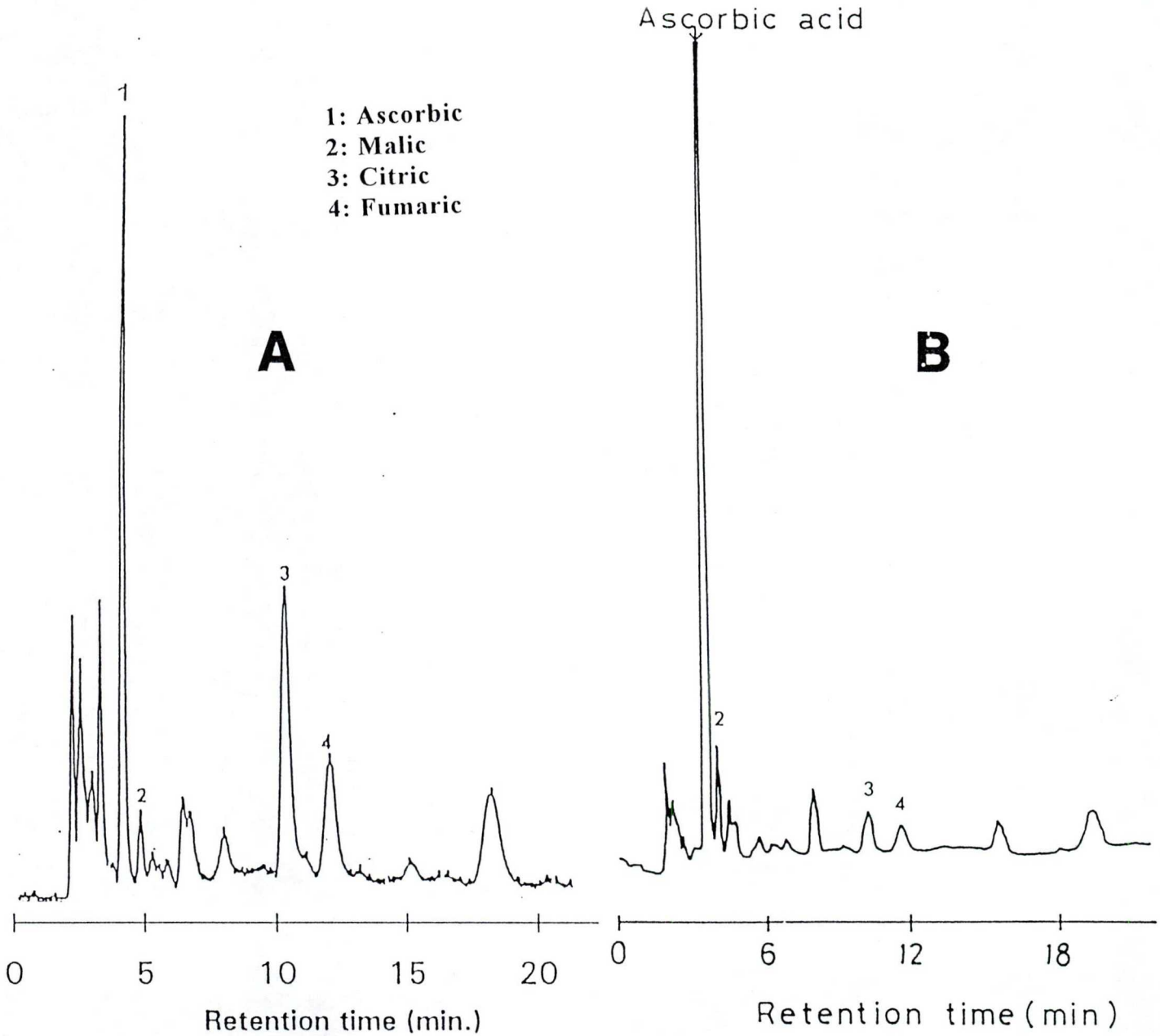


Figure 10 : HPLC profile of vitamin C and other organic acids extracted from (A) spice red pepper, (B) tomato.

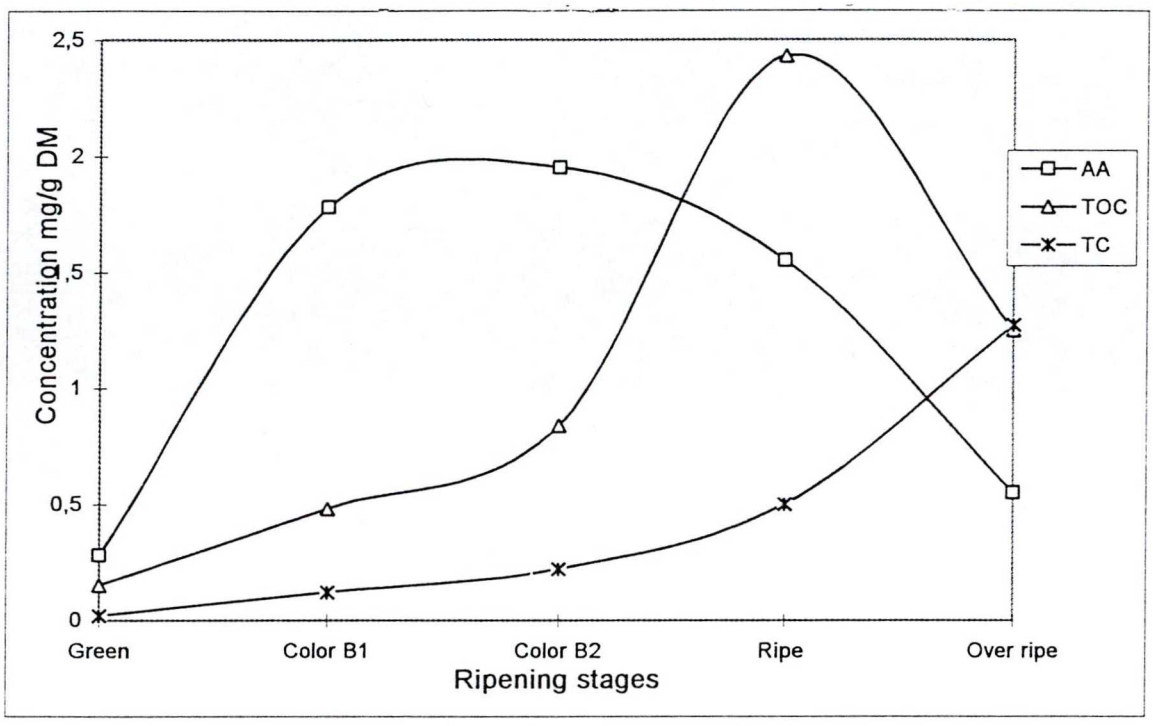


Figure 11 : Change in ascorbic acid (AA), tocopherol (TOC) and total carotenoids (TC) of spice red pepper (var. SZ-20) during ripening.

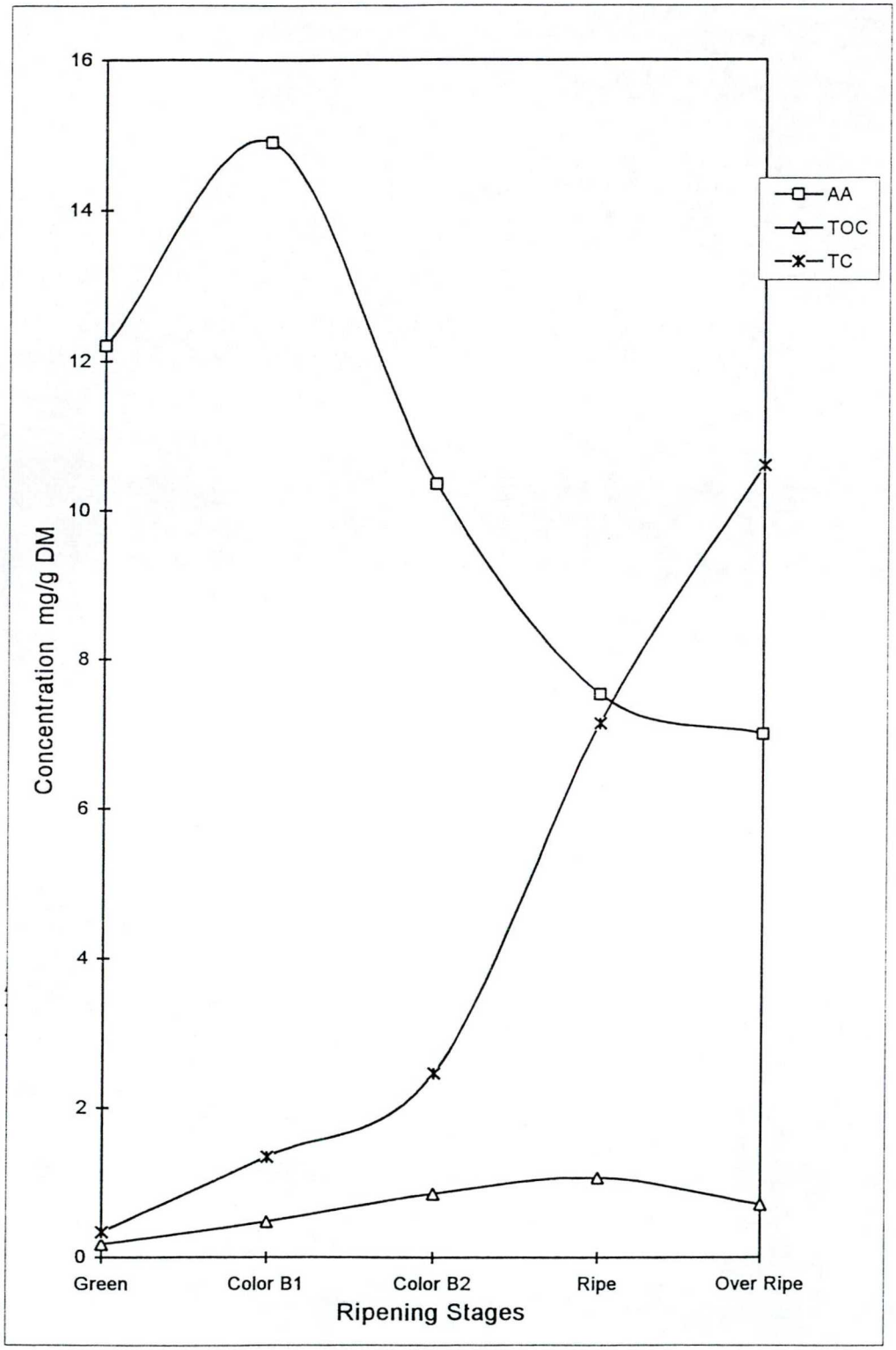


Figure 12 : Ascorbic acid (AA), tocopherol (TOC) and total carotenoids (TC) contents of spice red pepper (Km-622) during ripening stages.

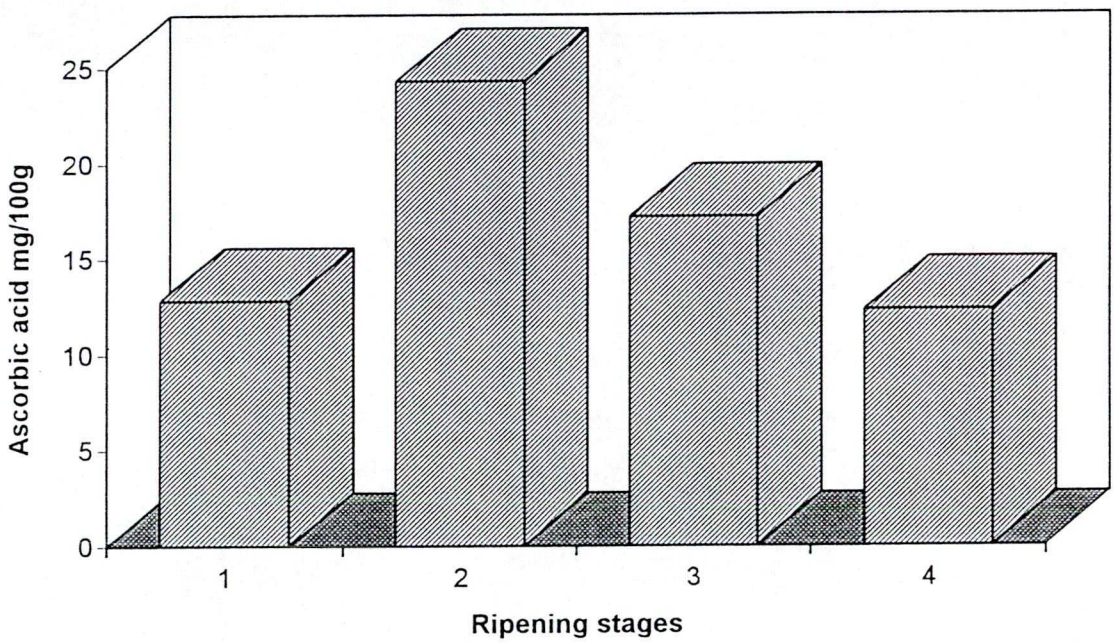


Figure 13 : Change in vitamin C content of tomato during ripening stages.

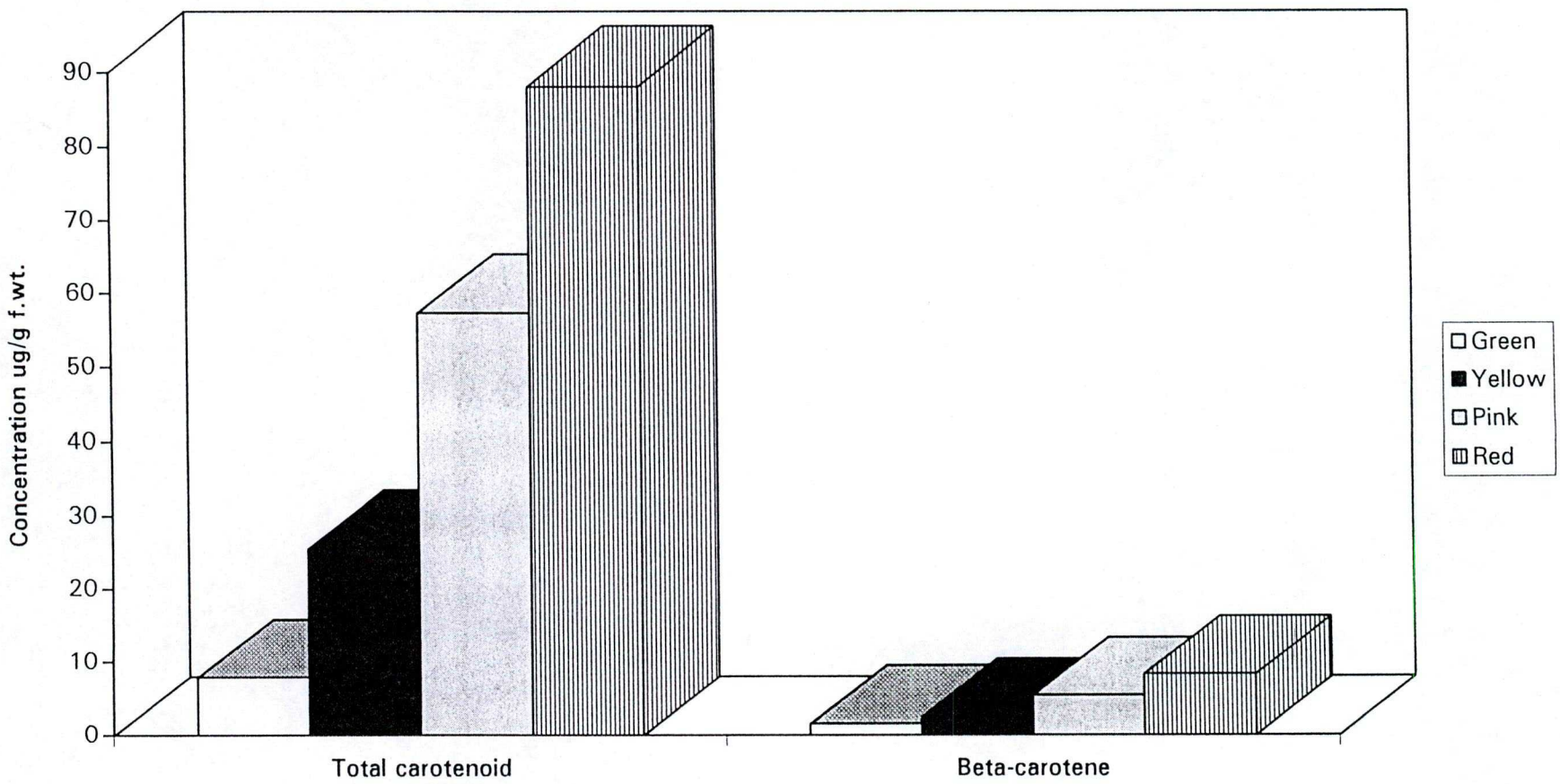


Figure 14 : Change in carotenoid content of tomato during ripening stages.

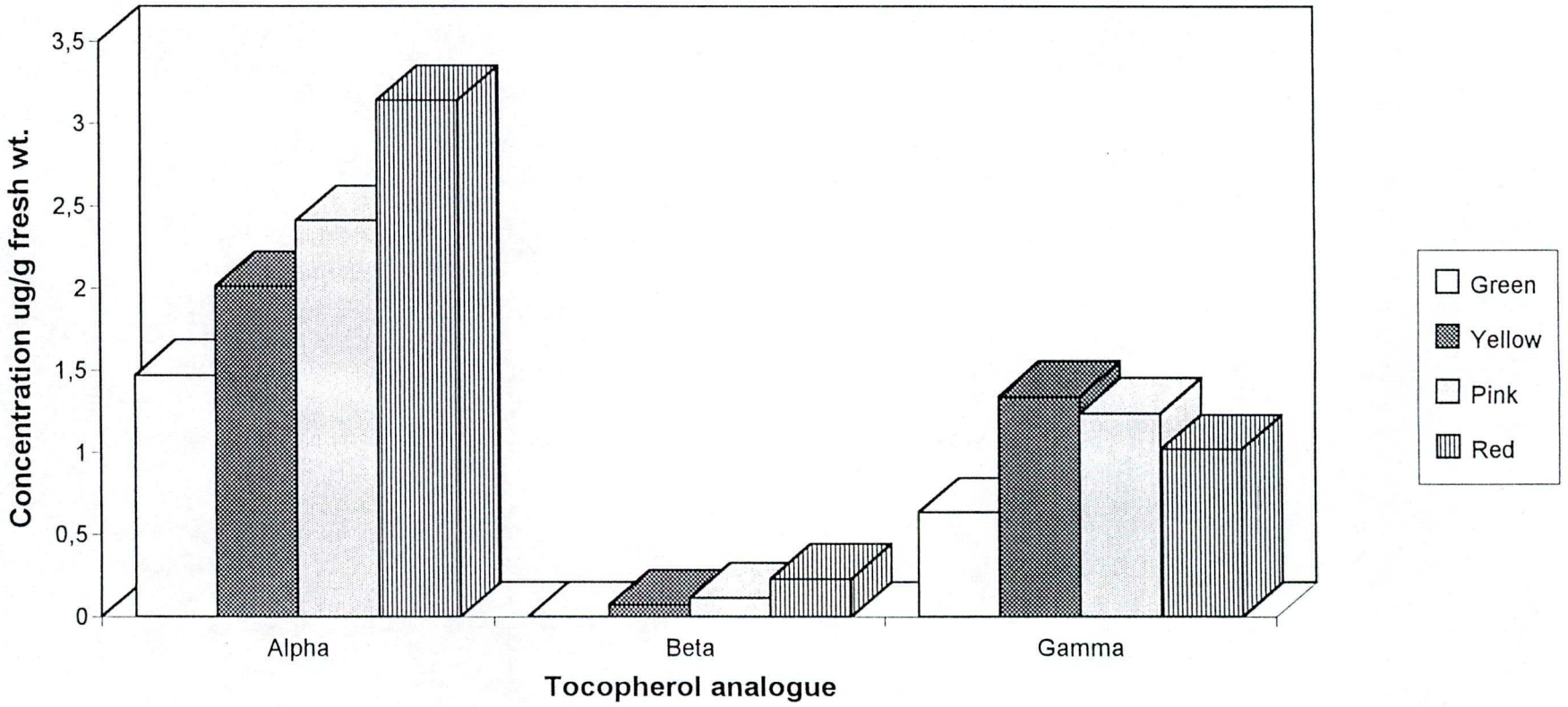


Figure 15 : Change in tocopherol content of tomato as a function of climacteric ripening.

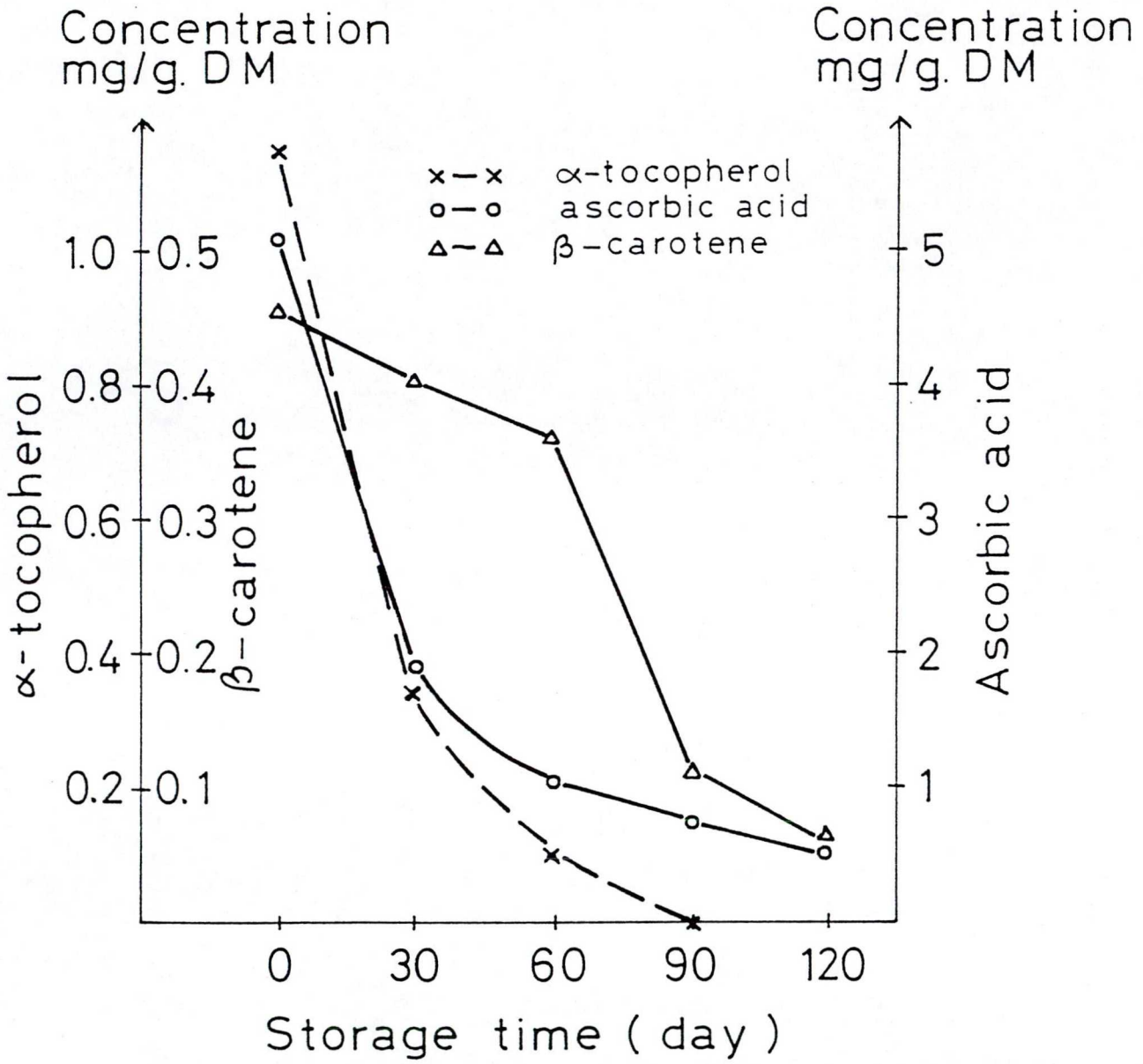


Figure 16 : Effect of ambient storage on the antioxidant content of ground paprika (var. Km-622).

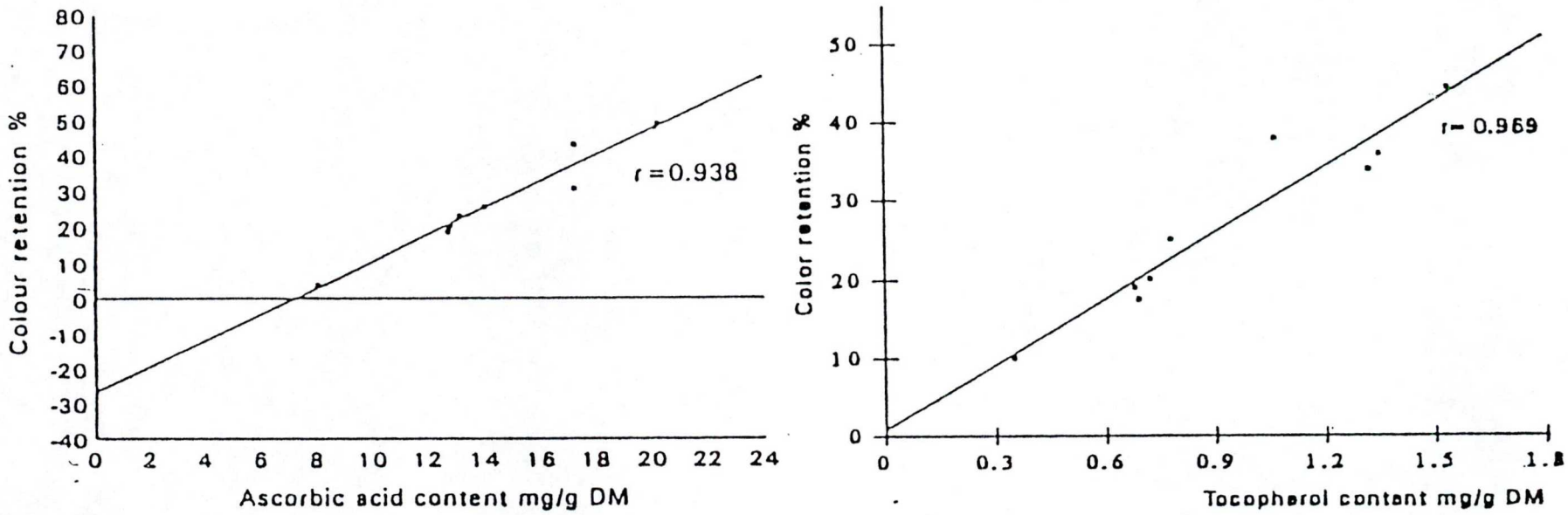


Figure 17 : Correlation between antioxidants and color retention by spice red pepper powder.

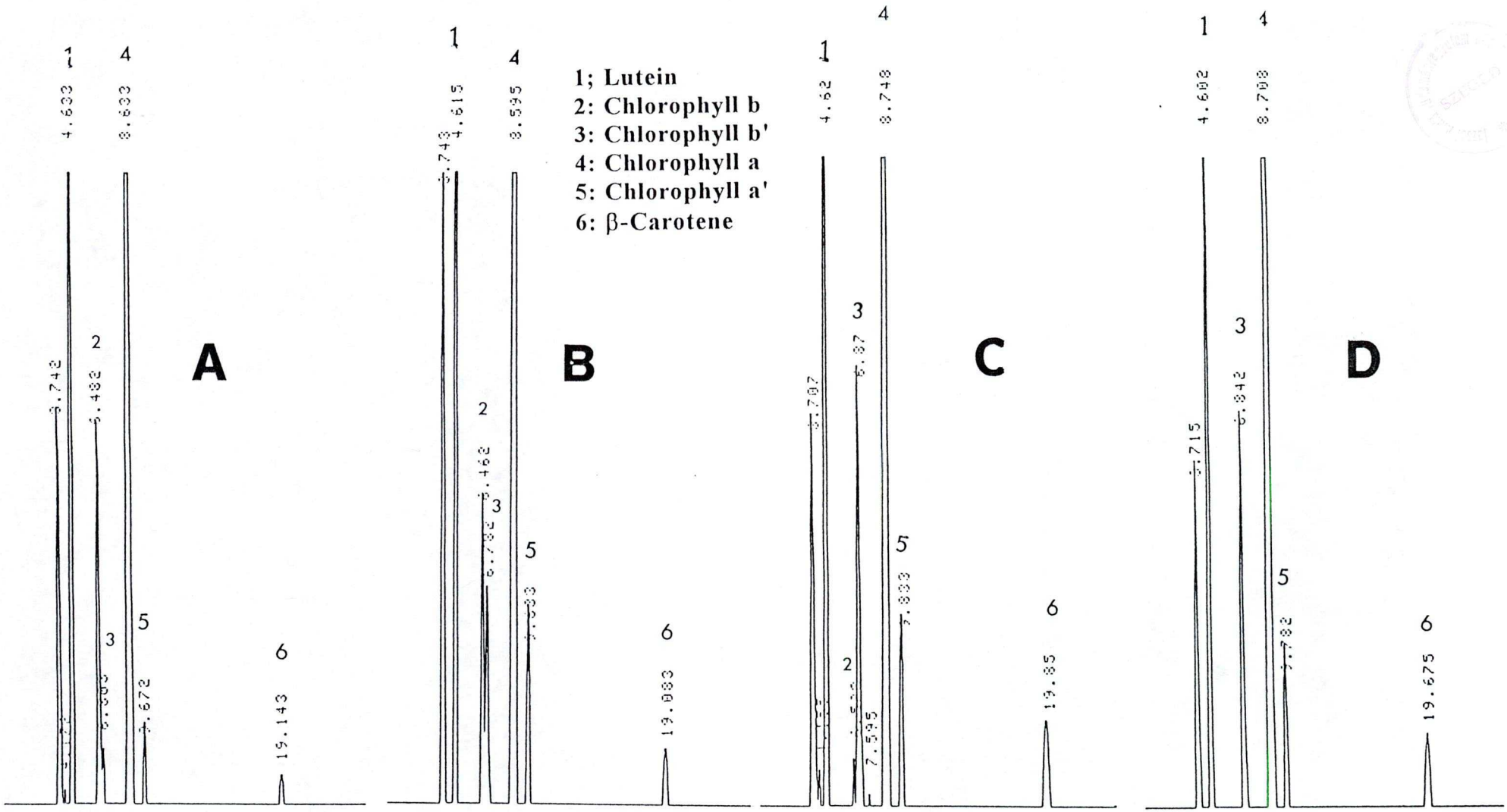


Figure 18:Effect of Cr treatments on photosynthetic pigment composition in spanich leaves. (A) control , (B) 90 kg/ha , (C) 270 kg/ha, (D) 810 kg/ha.

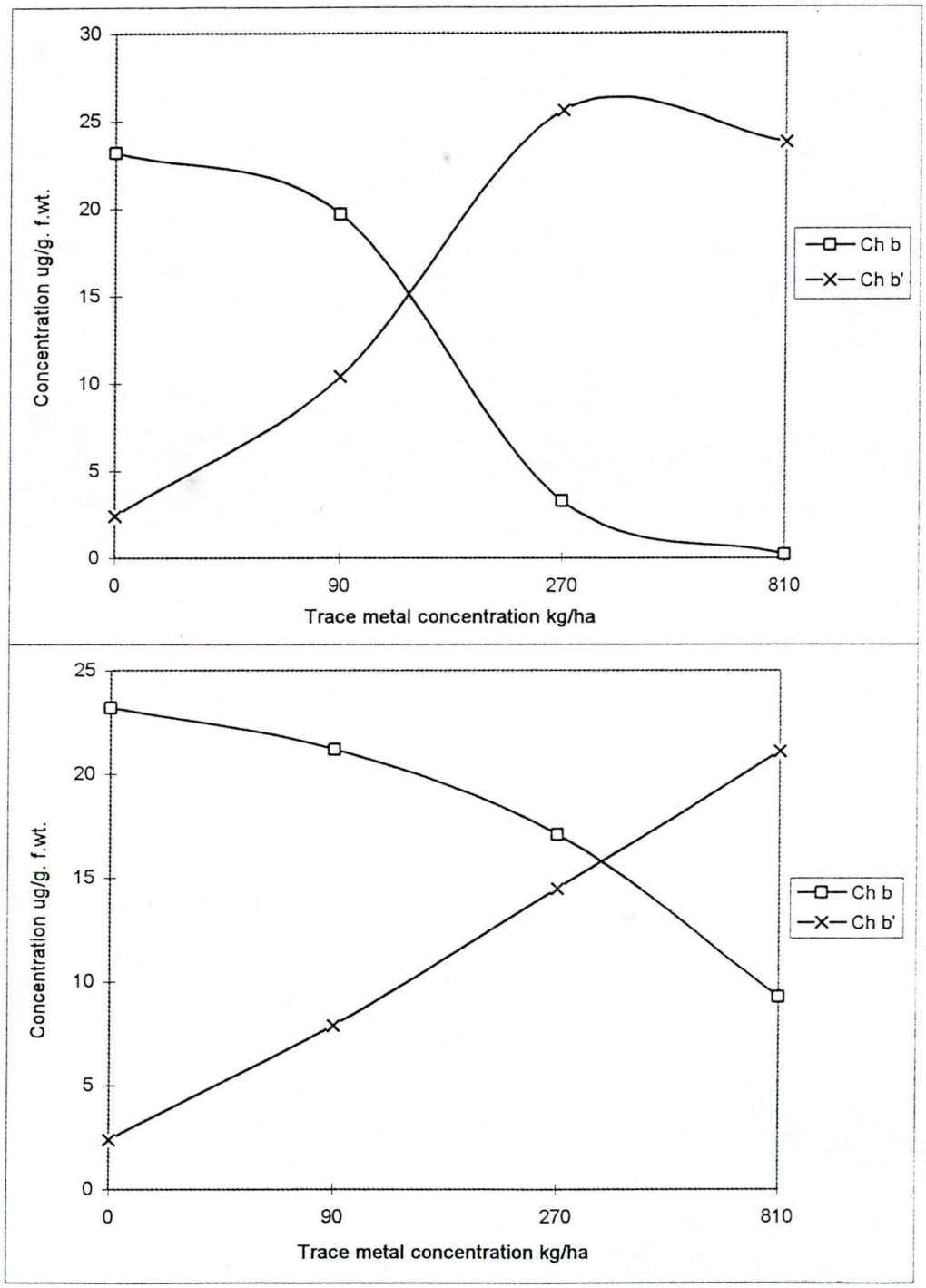


Figure 19 : Change in chlorophyll b and chlorophyll b' content of spinach leaves as a function of (A) Cr- and (B) Mo-load in soil.

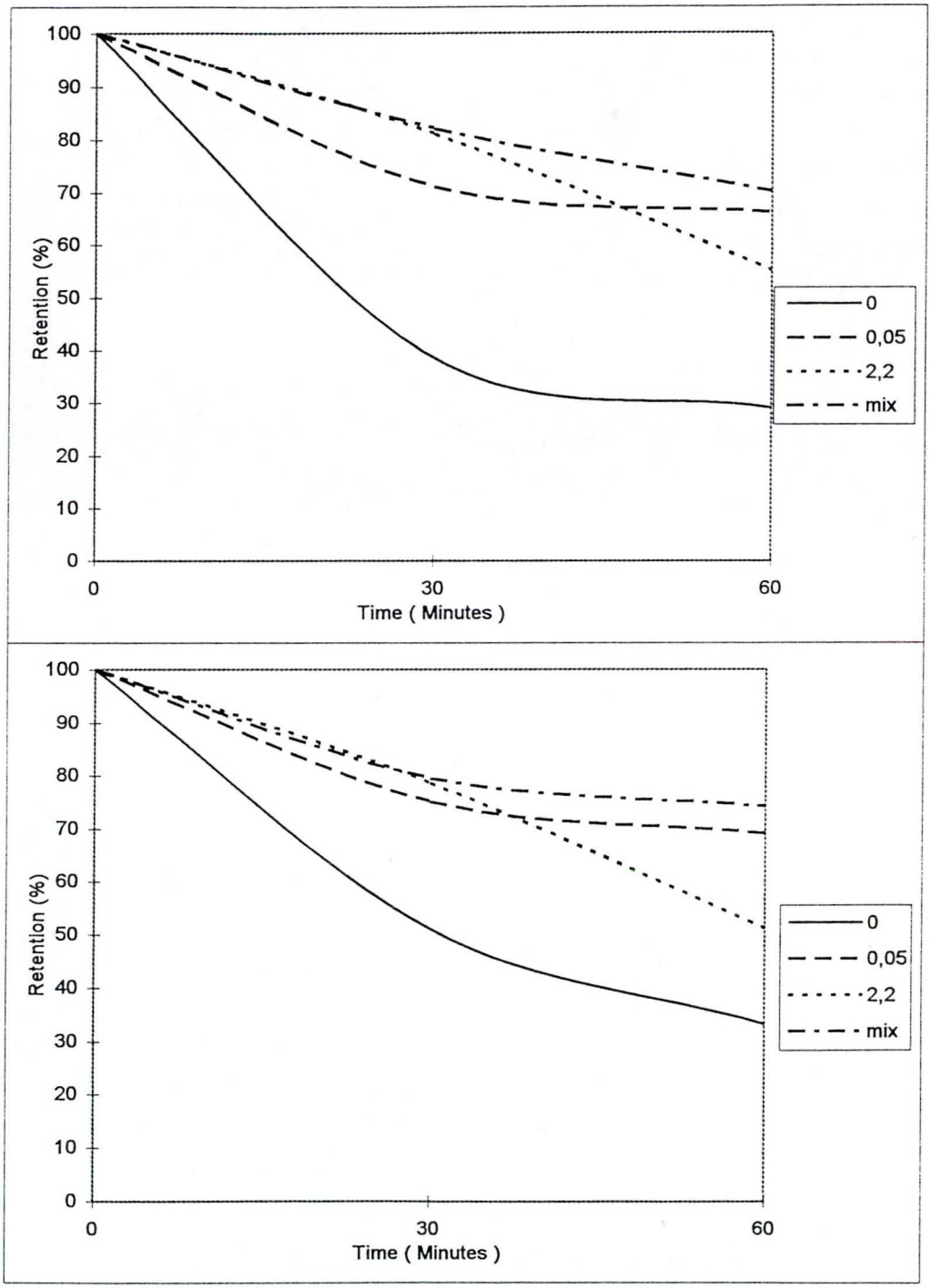


Figure 20 : LOX-catalyzed cooxidation of (A) chlorophyll a and (B) chlorophyll b from parsley extract in presence of a mixture of 0.05 mg/ml ascorbic acid and 2.2 mg/ml ∝ -tocopherol acetate.

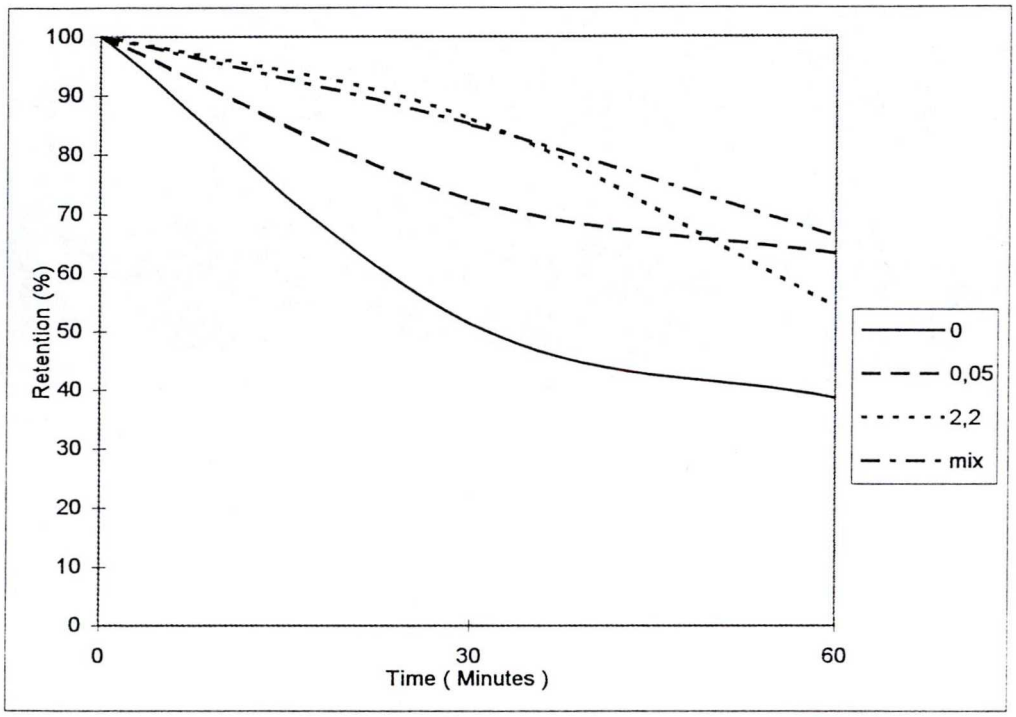


Figure 21 : LOX-catalyzed cooxidation of β-carotene from parsley extract in presence of a mixture of 0.05 mg/ml ascorbic acid and 2.2 mg/ml α-tocopherol acetate.

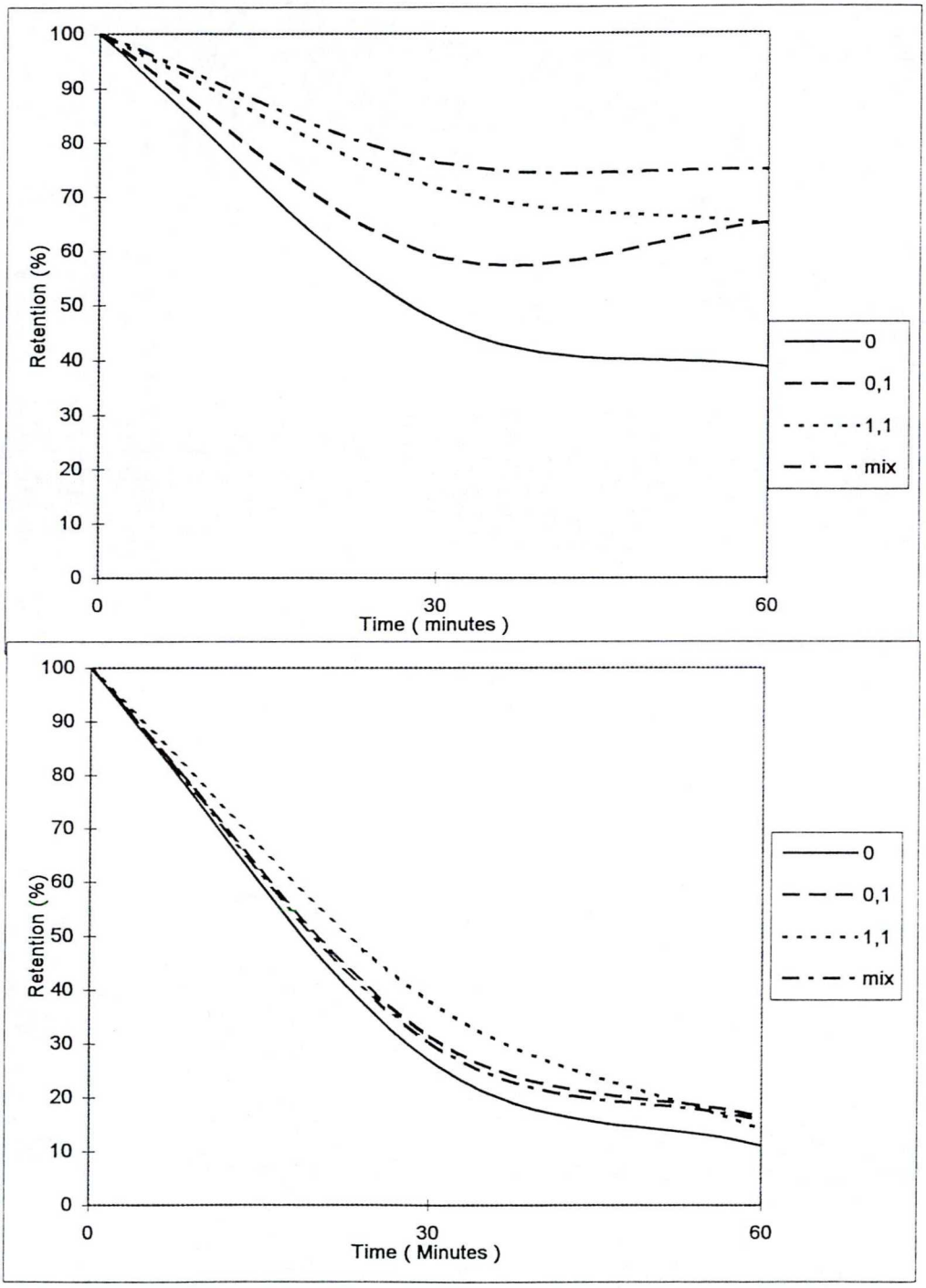


Figure 22 : LOX-catalyzed cooxidation of (A) lycopene and (B) β-carotene from tomato extract in presence of a mixture of 0.1 mg/ml ascorbic acid and 1.1 mg/ml α-tocopherol acetate.

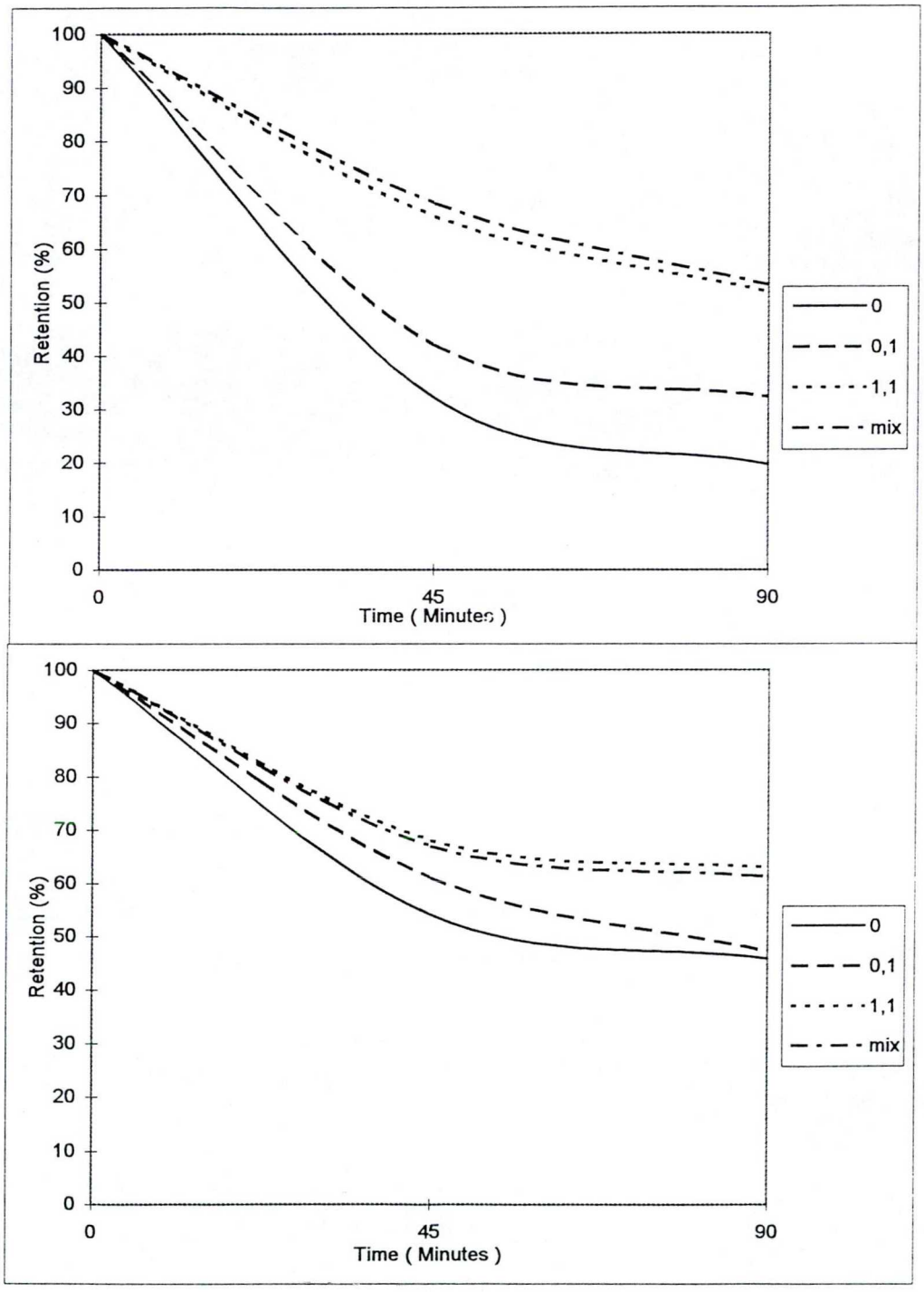


Figure 23 : LOX-catalyzed cooxidation of (A) CME and (B) CDE from spice red pepper in presence of a mixture of 0.1 mg/ml ascorbic acid and 1.1 mg/ml α-tocopherol acetate.

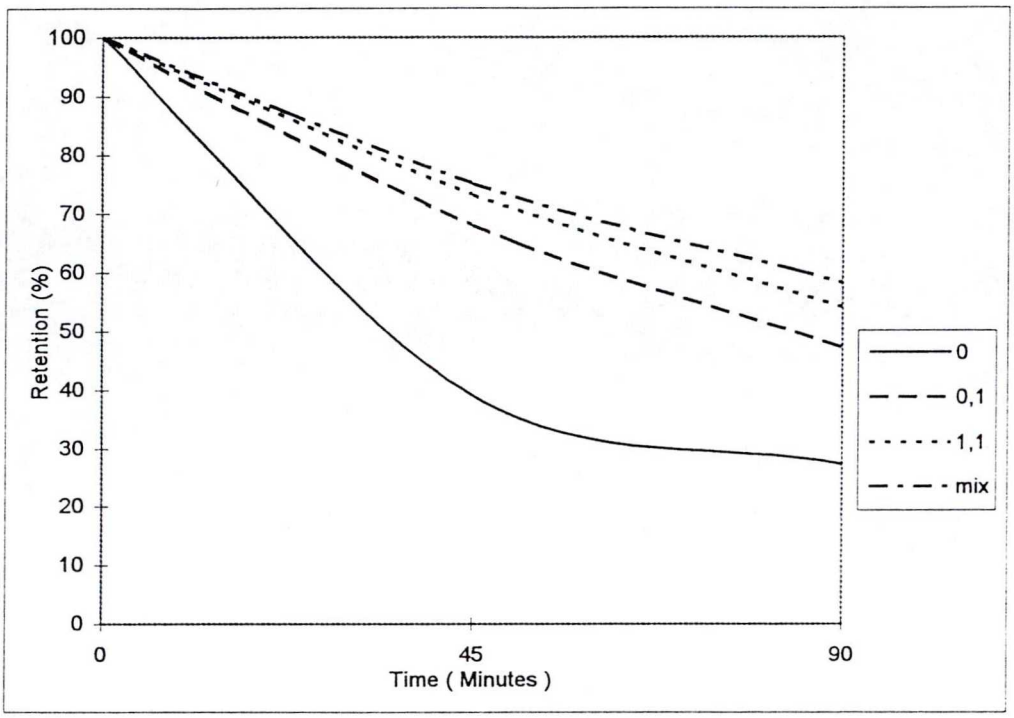


Figure 24 : LOX-catalyzed cooxidation of β-carotene from spice pepper in presence of a mixture of 0.1 mg/ml ascorbic acid and 1.1 mg/ml α-tocopherol acetate.

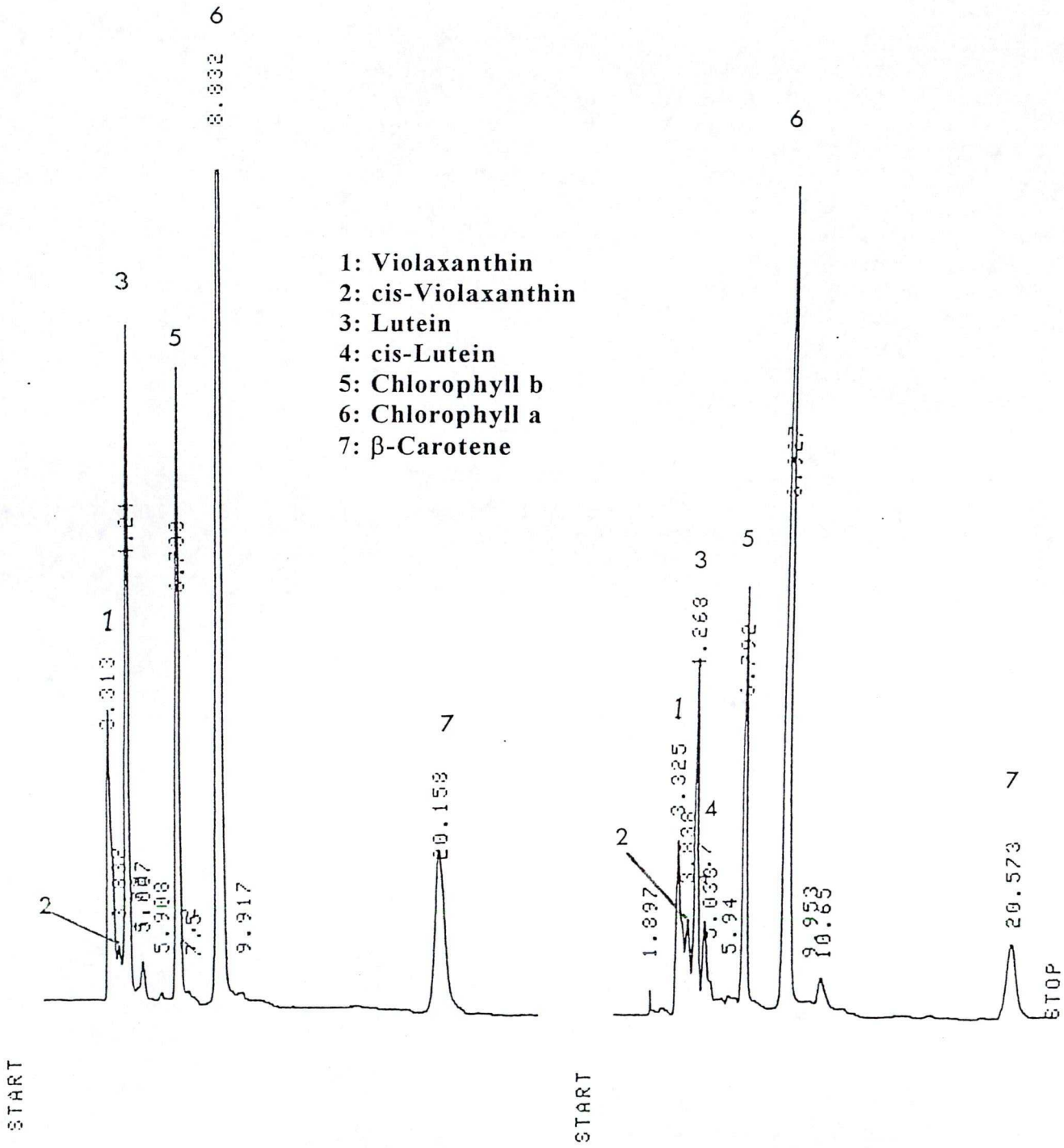


Figure 25 : HPLC profile of photosynthetic pigments of parsley extract after 0 min (left) and 60 min (right) of exposure to light in the model system.

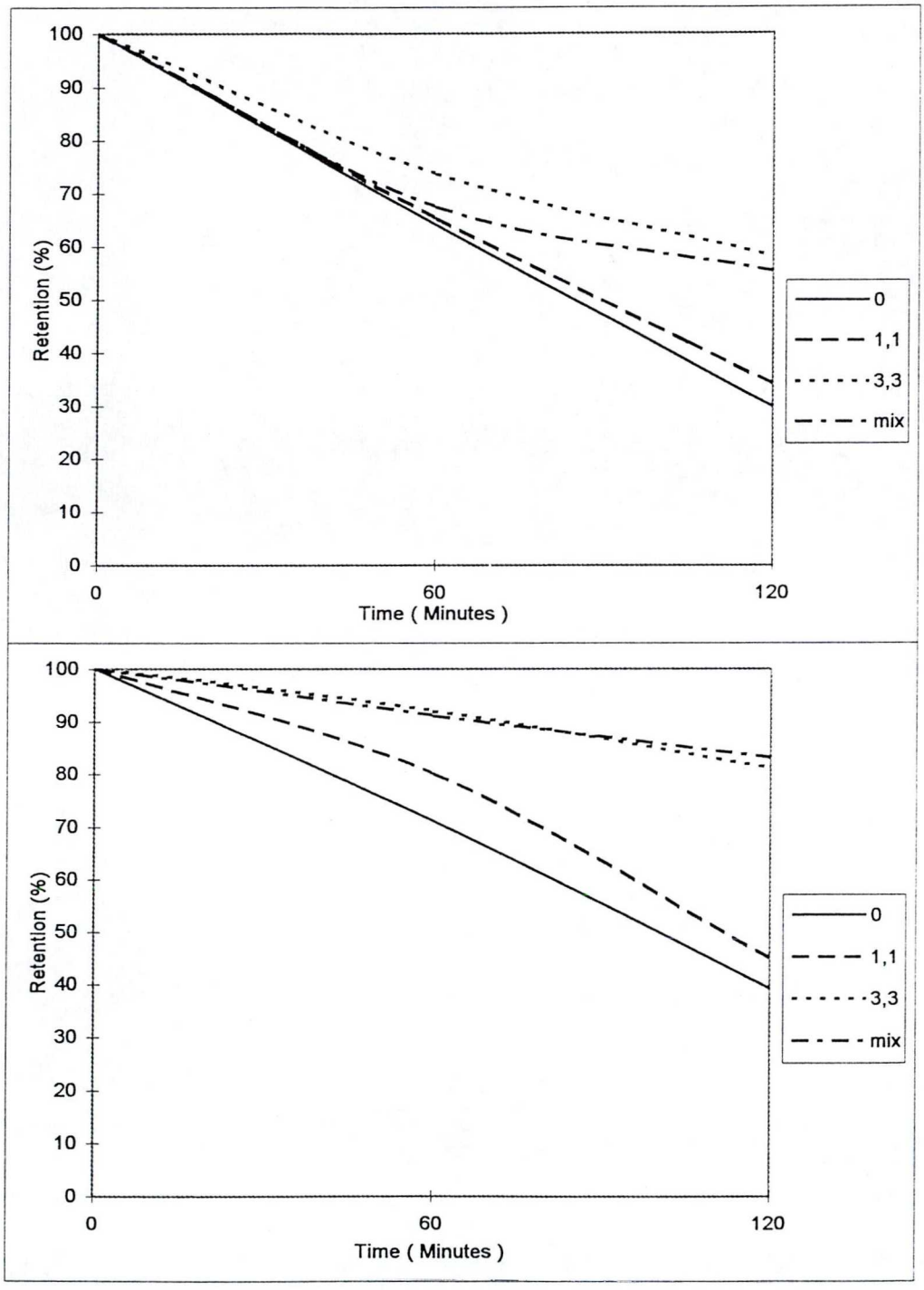


Figure 26 : Photooxidation of (A) chlorophyll a and (B) chlorophyll b from parsley extract in presence of 1.1 mg/ml ascorbyl palmitate and 3.3 mg/ml α-tocopherol.

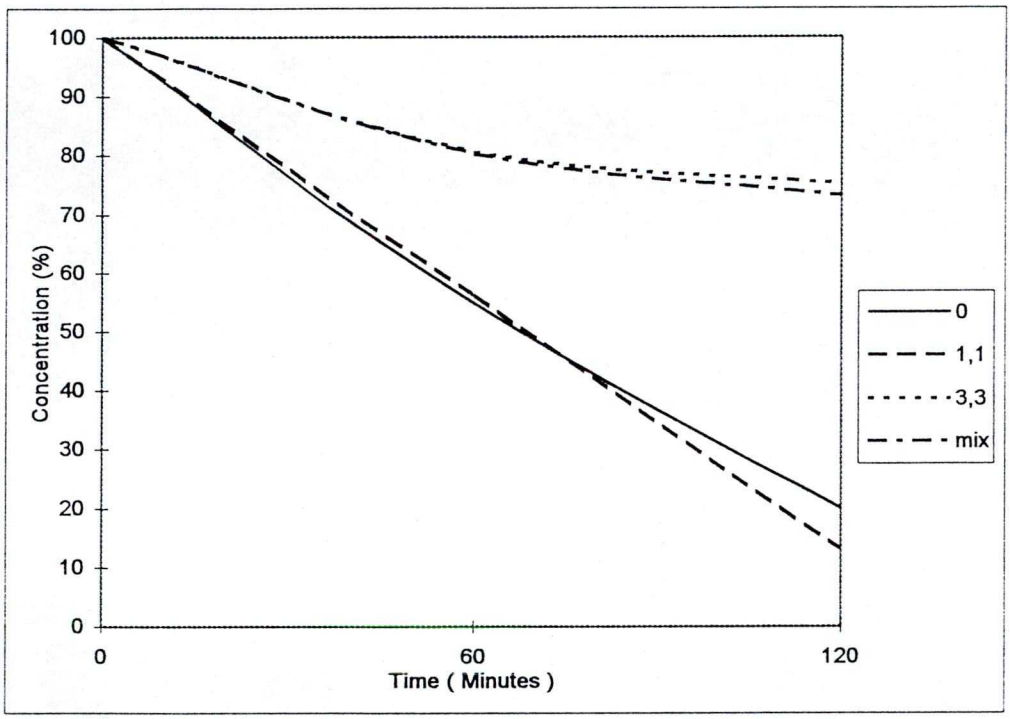


Figure 27 : Photooxidation of β-carotene from parsley extract in presence of a mixture of 1.1 mg/ml ascorbyl palmitate and 3.3 mg/ml α-tocopherol.

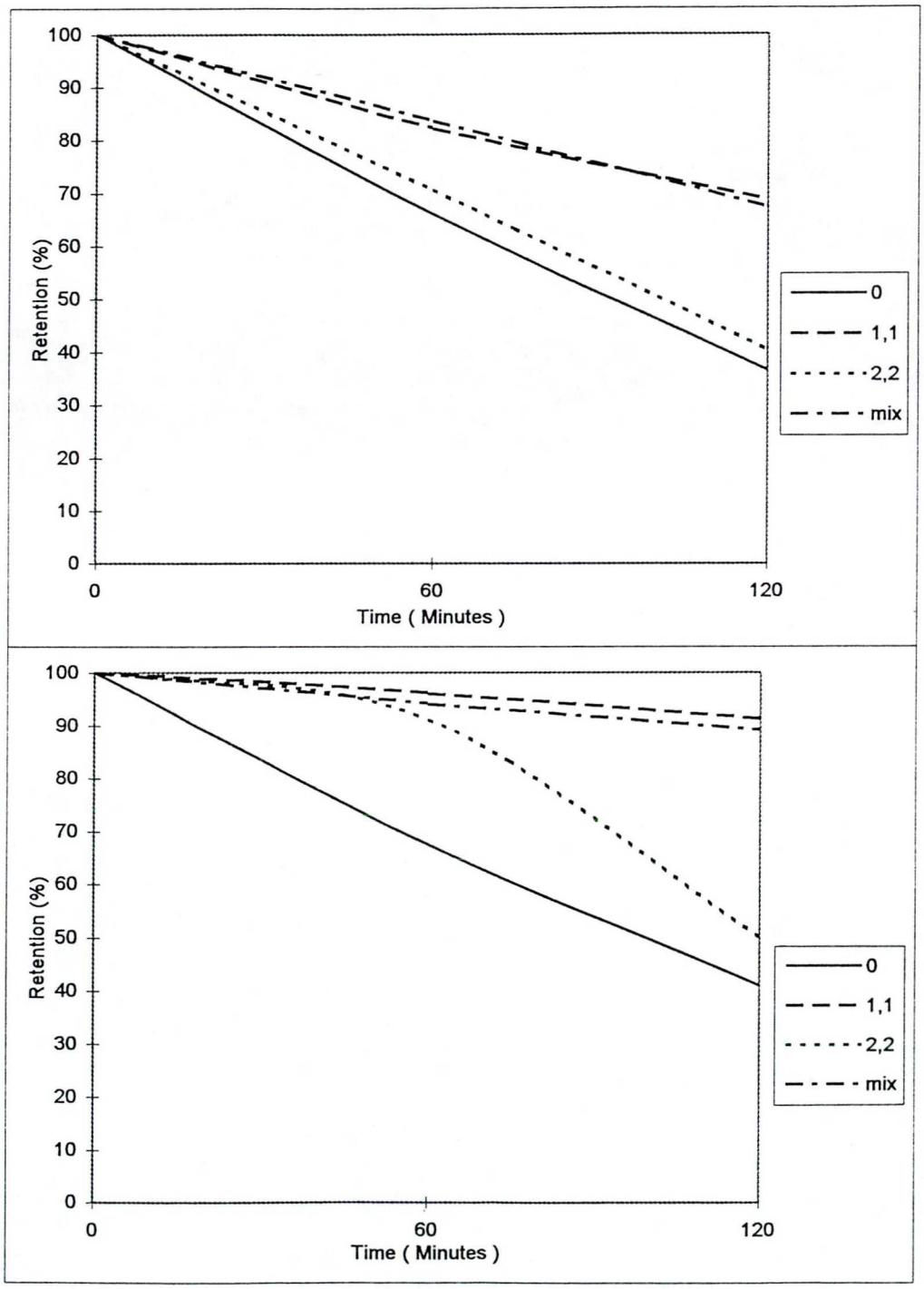


Figure 28 : Photooxidation of (A) lycopene and (B) β-carotene tomato extract in presence of a mixture of 1.1 mg/ml ∝-tocopherol and 2.2 mg/ml ascorbyl palmitate.

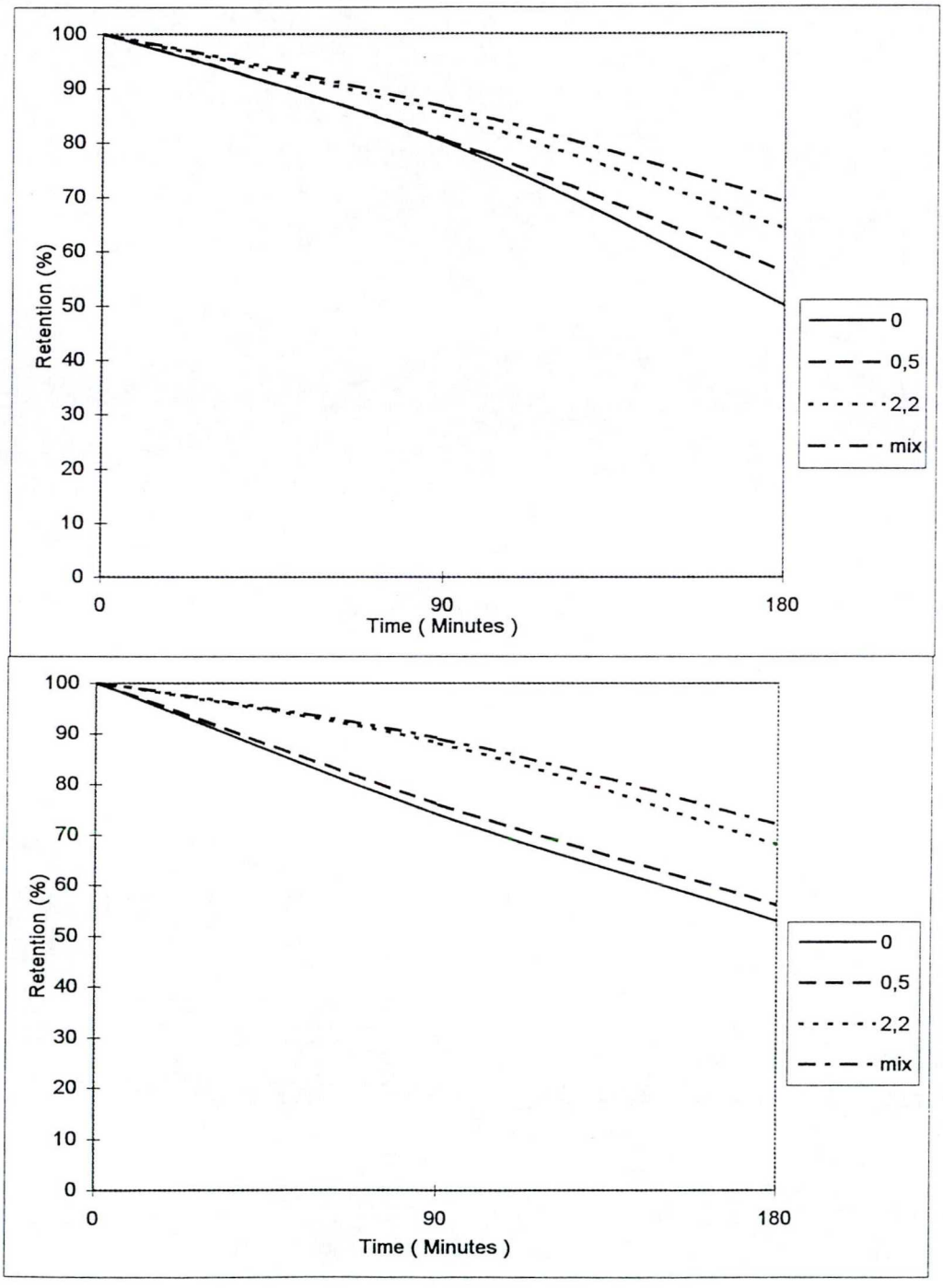


Figure 29 : Photooxidation of (A) CME and (B) CDE from spice red pepper in presence of a mixture of 0.5 mg/ml α-tocopherol and 2.2 mg/ml ascorbyl palmitate.

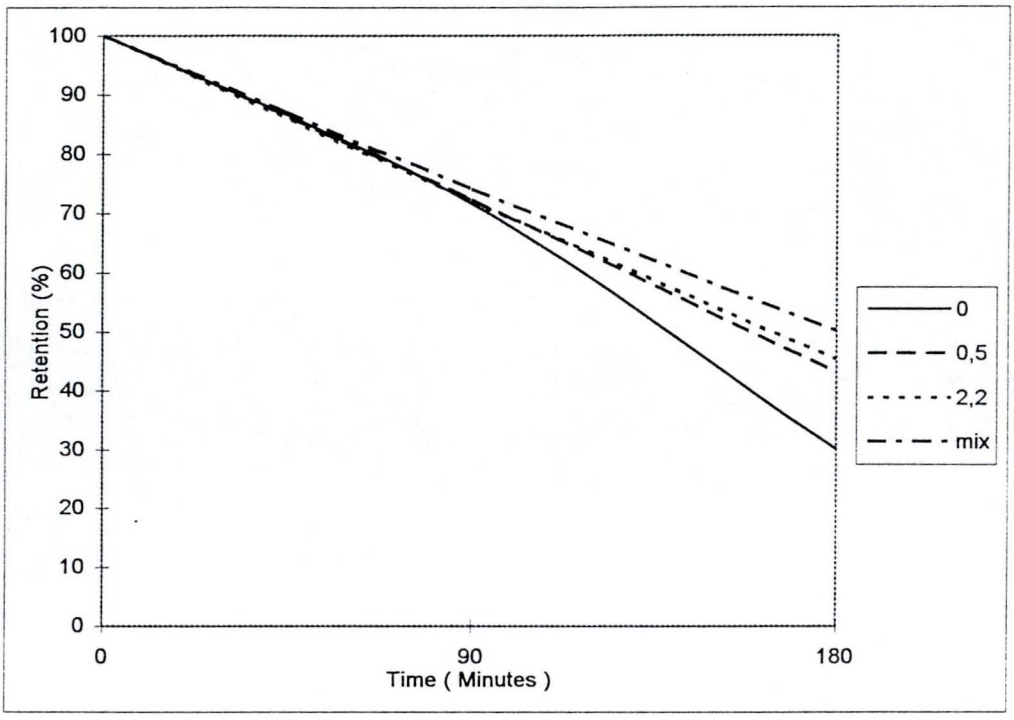


Figure 30 : Photooxidation of β-carotene from spice red pepper presence of a mixture of 0.5 mg/ml α -tocopherol and 2.2 mg/ml ascorbyl palmitate.

10. APPENDIX

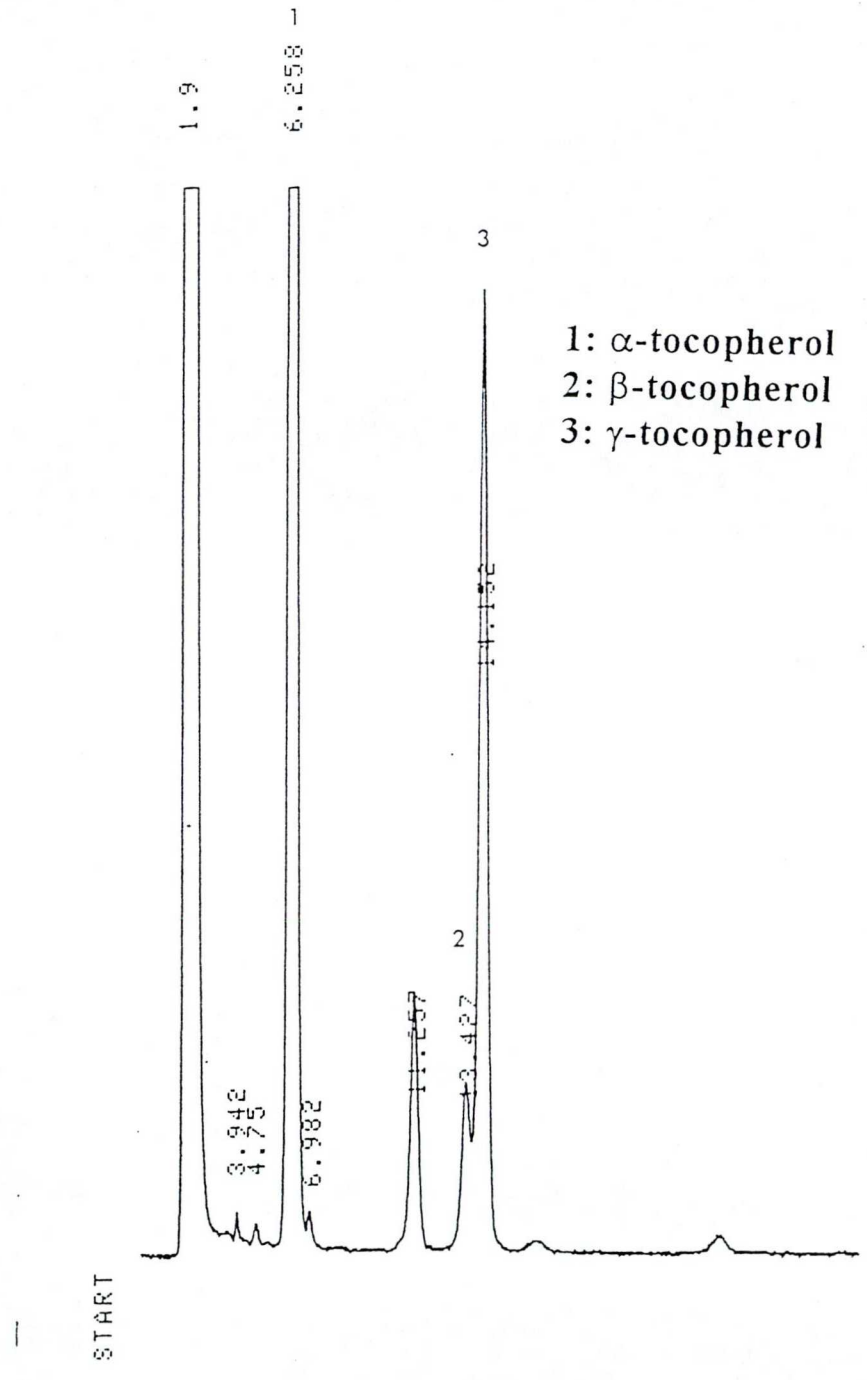


Figure 1 : HPLC separation of tocopherol analogues from spice red pepper (with seeds).

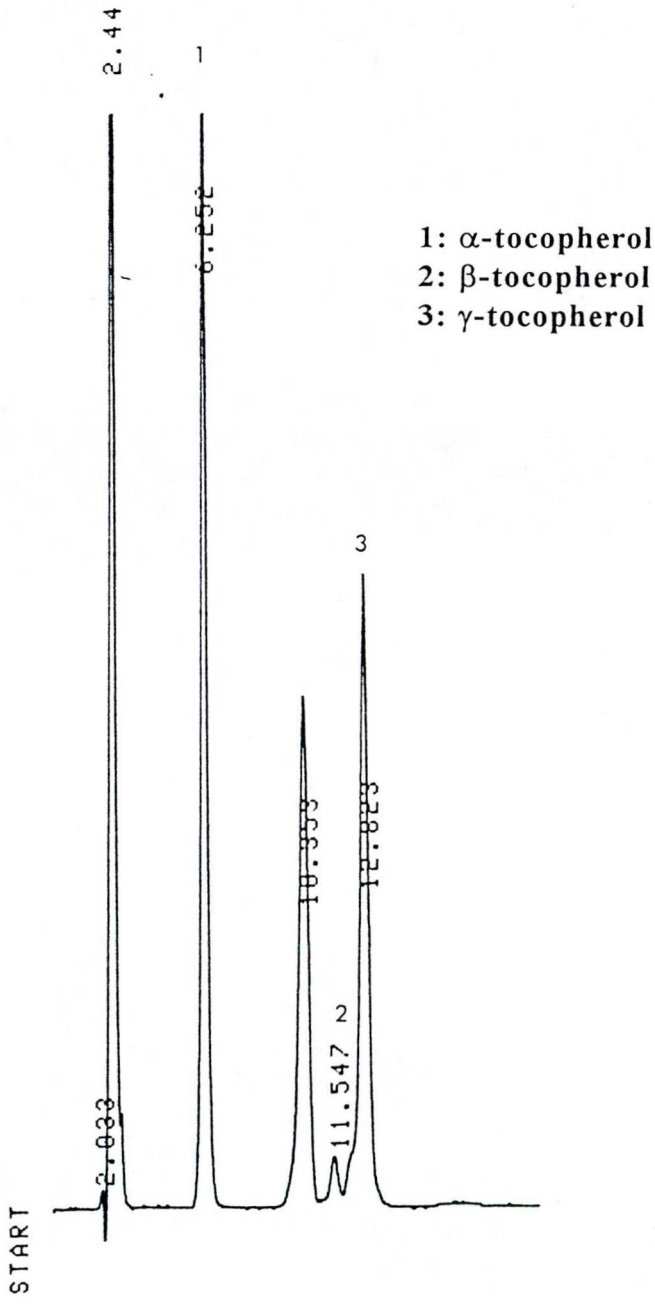


Figure 2 : HPLC separation of tocopherol analogues from ripe tomato.

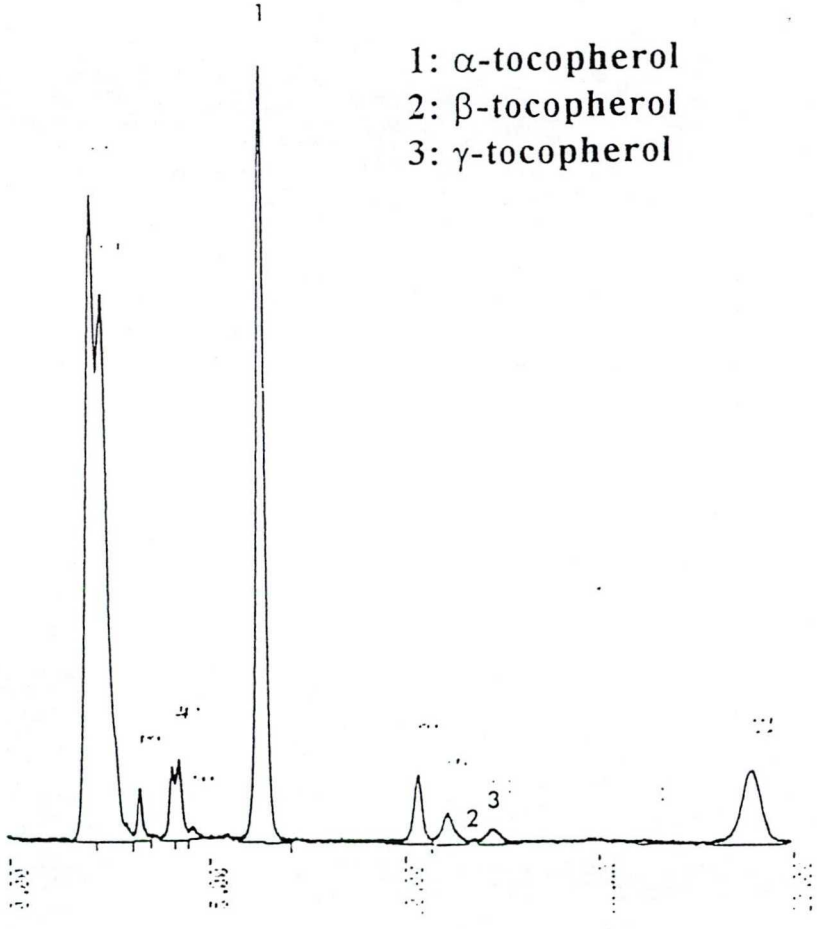


Figure 3 : HPLC separation of tocopherol analogues from parsley.

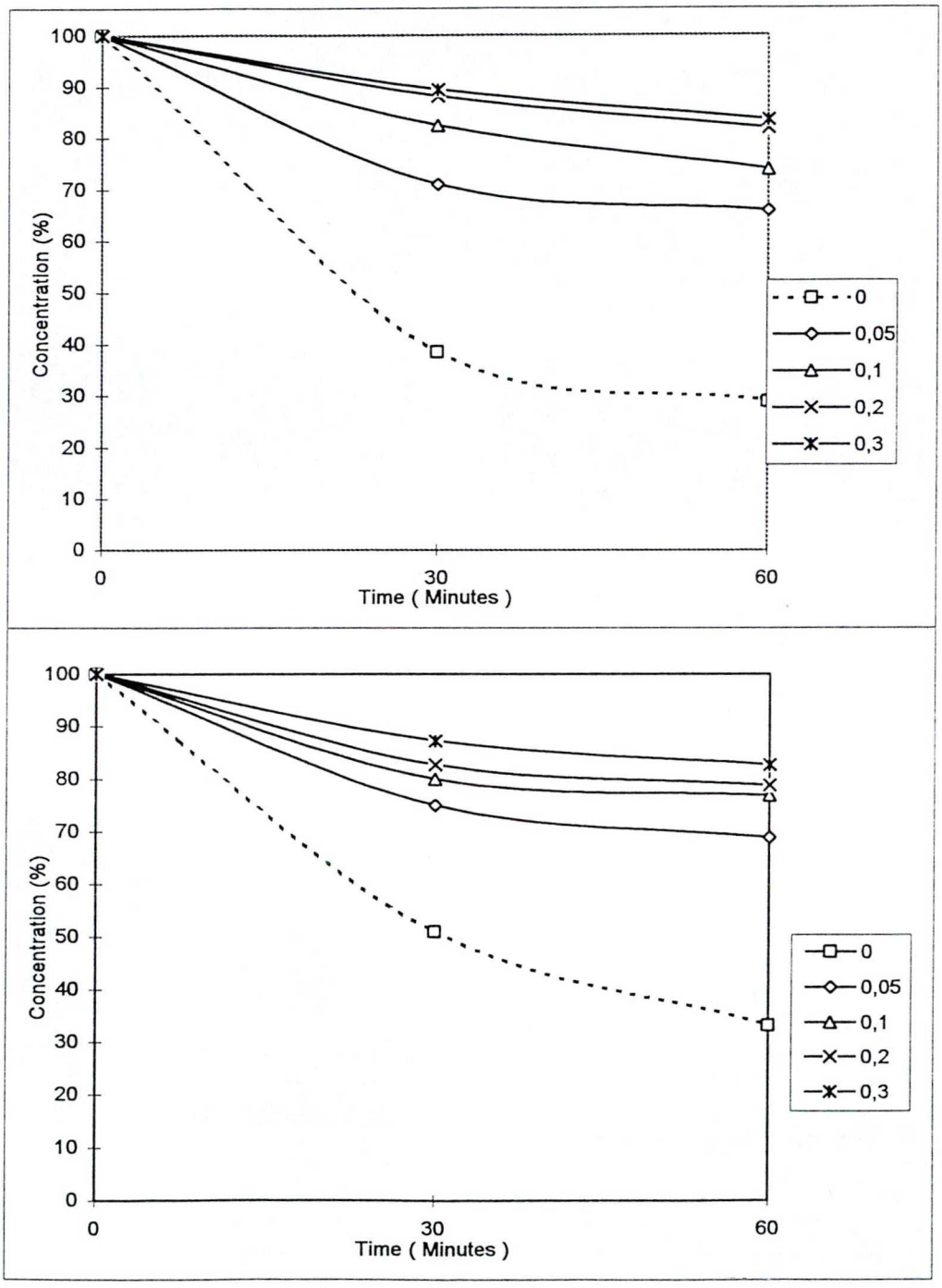


Figure 4 : Lox-catalyzed cooxidation of (A) chlorophyll a and (B) chlorophyll b from parsely extract in presence of ascorbic acid at different concentrations.

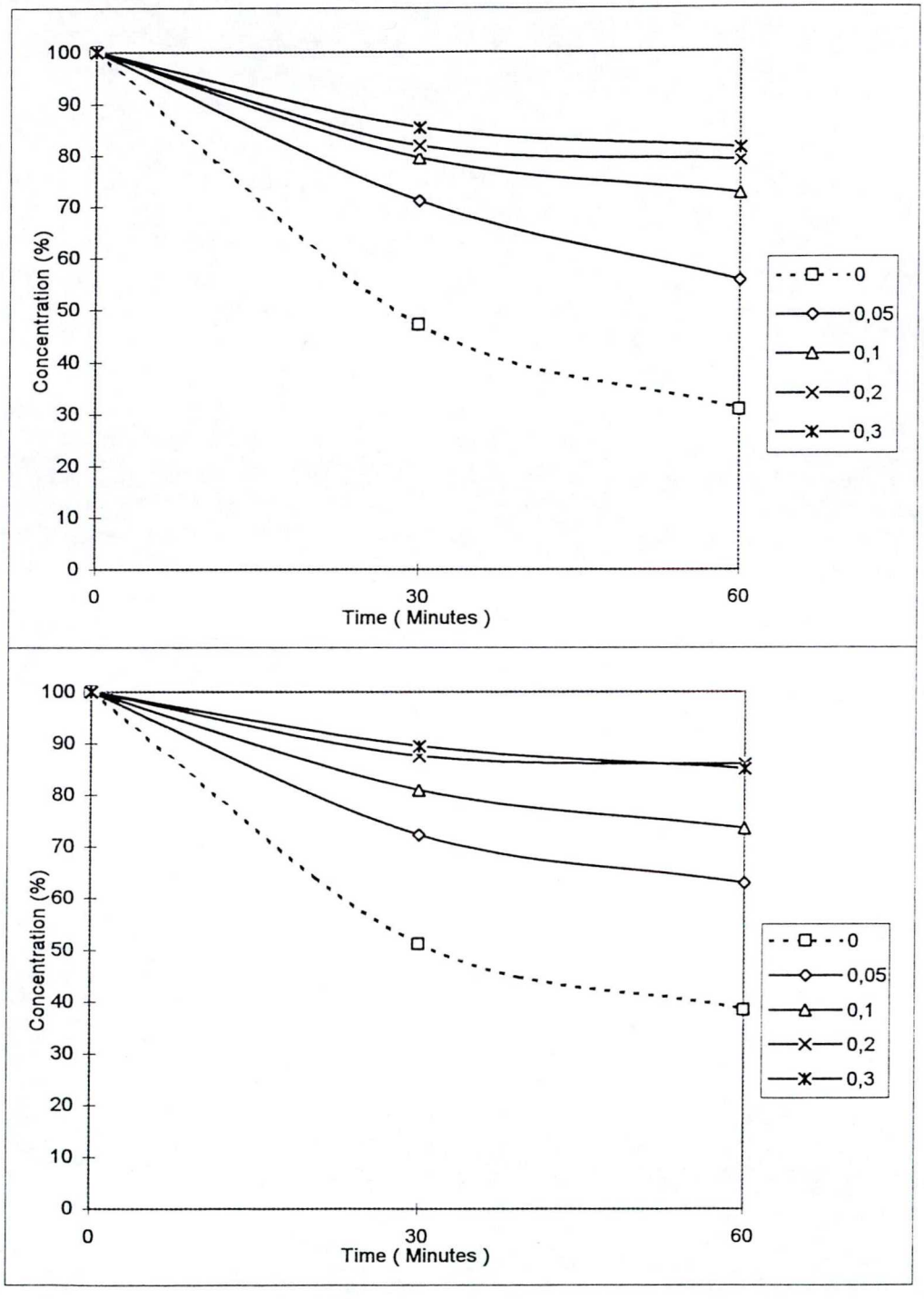


Figure 5 : Lox-catalyzed cooxidation of (A) lutein and (B) b-carotene from parsely extract in presence of ascorbic acid at different concentrations.

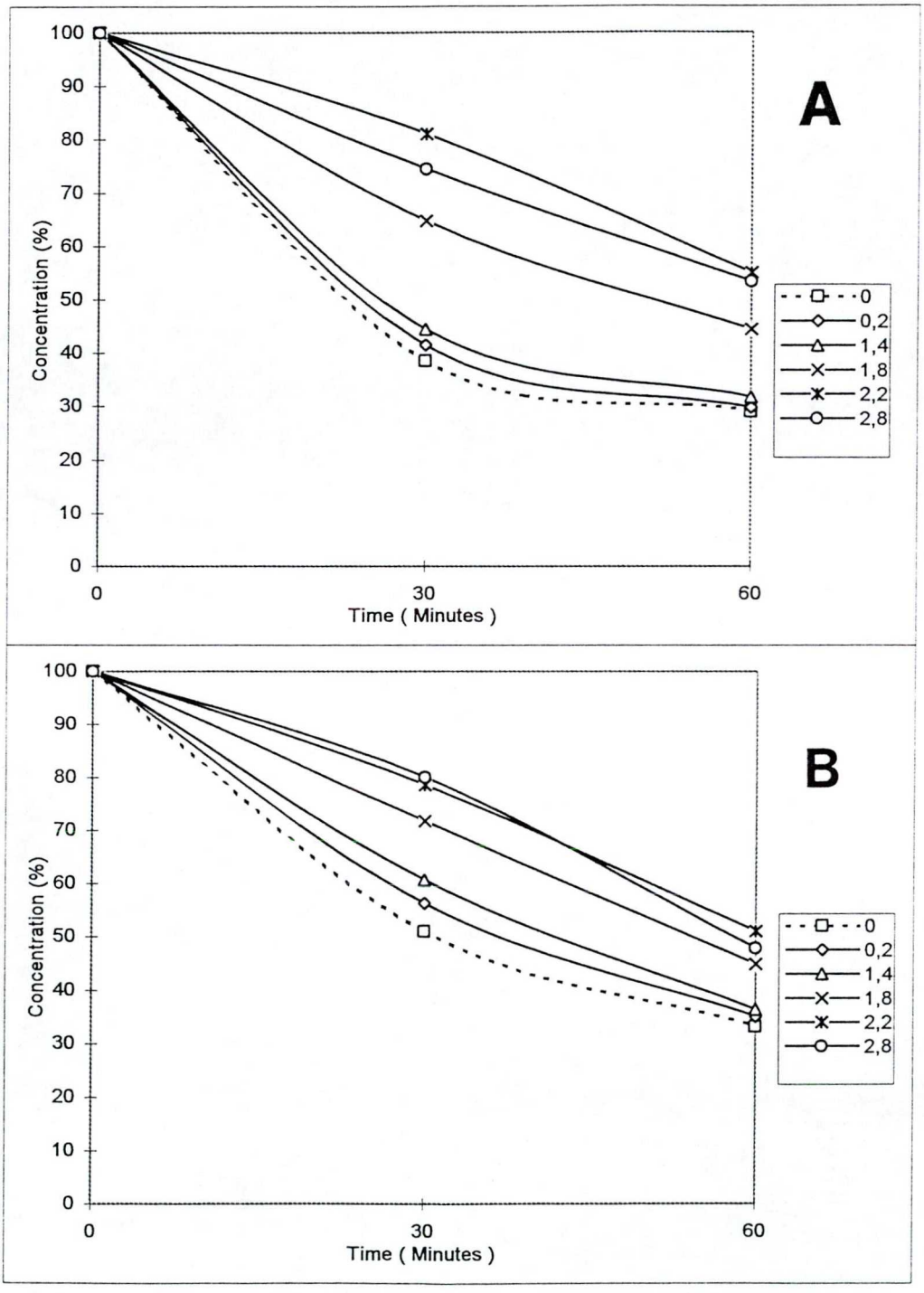


Figure 6 : Lox-catalyzed cooxidation of (A) chlorophyll a and (B) chlorophyll b from parsely extract in presence of ∝-tocopherol acetate at different concentrations.

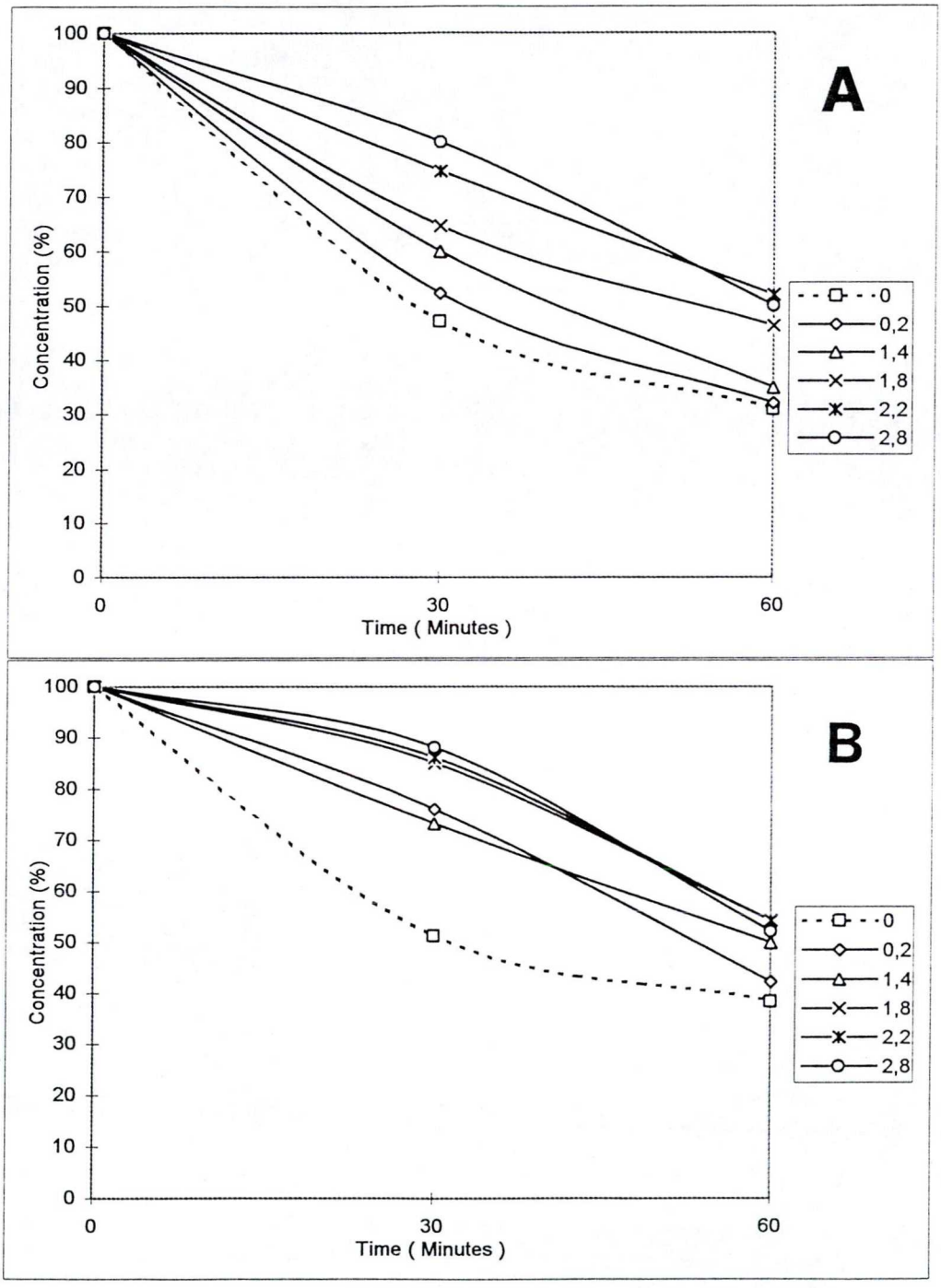


Figure 7 : Lox-catalyzed cooxidation of (A) lutein and (B) B-carotene from parsely extract in presence of∝-tocopherol acetate at different concentrations.

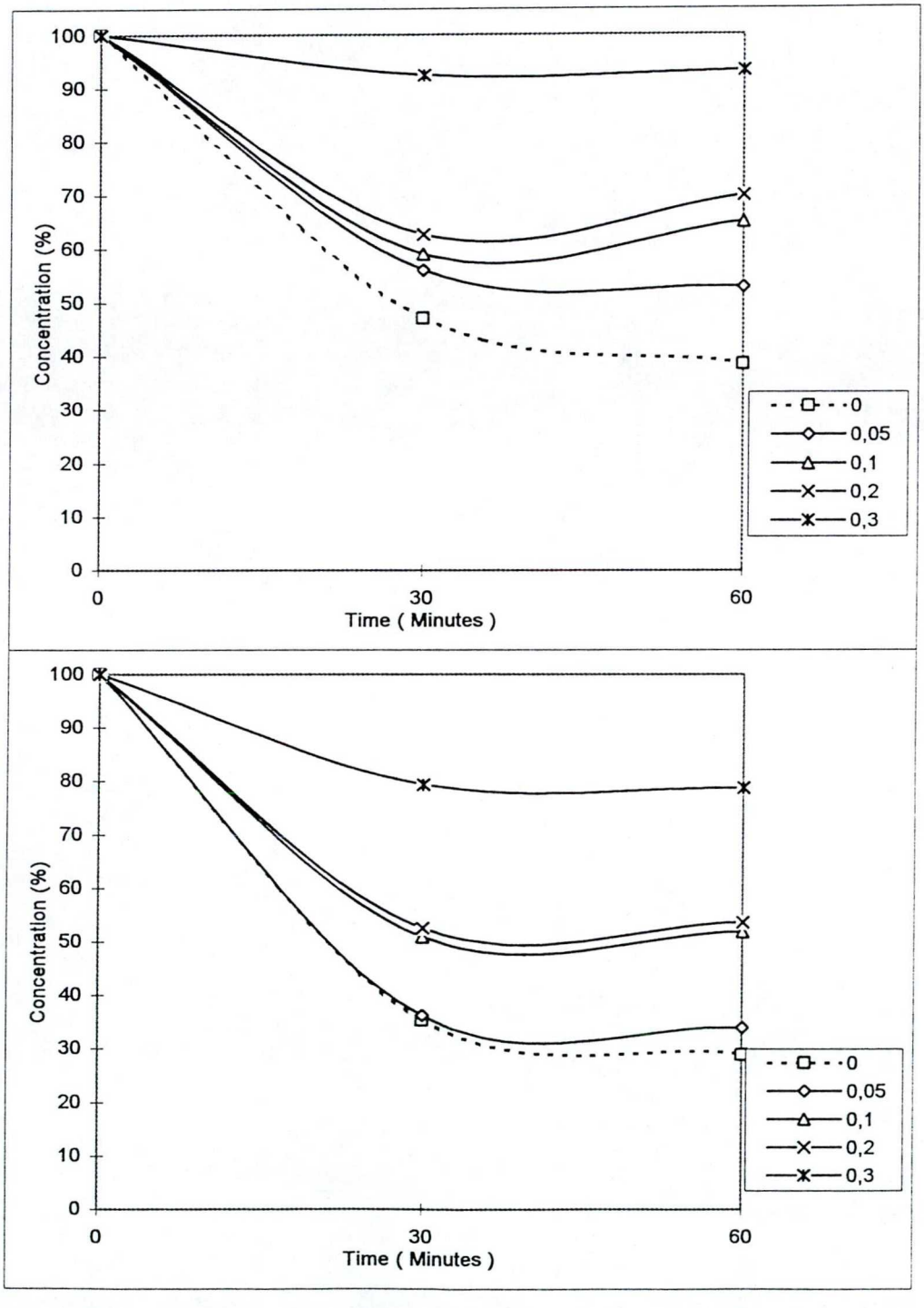


Figure 8 : Lox-catalyzed cooxidation of (A) lycopene and (B) lycoxanthin from tomato extract in presence of ascorbic acid at different concentrations.

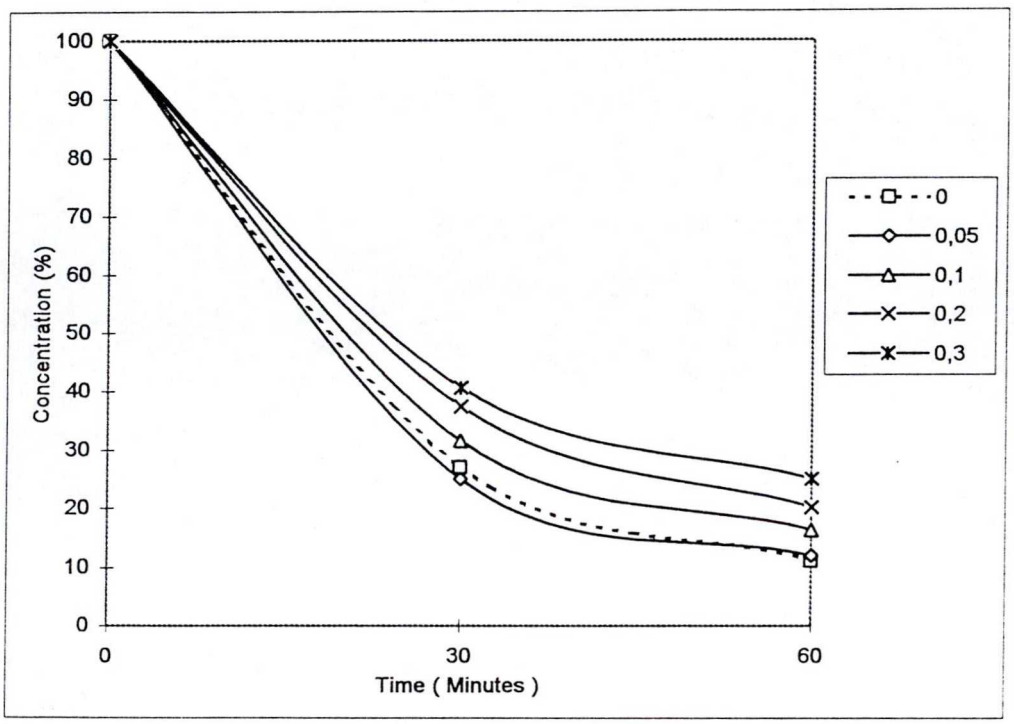


Figure 9 : Lox-catalyzed cooxidation of B-carotene from tomato extract in presence of ascorbic acid at different concentrations.

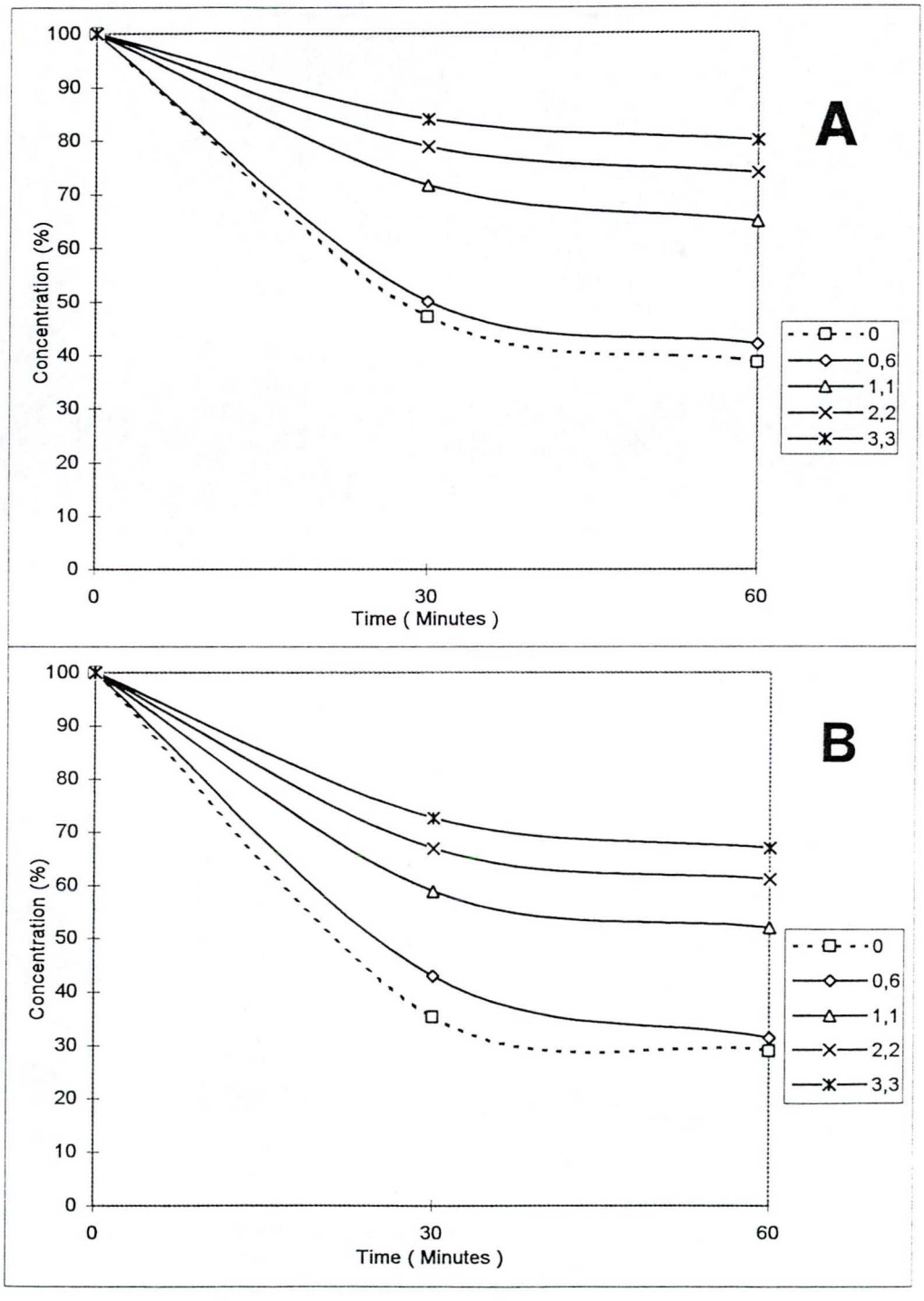


Figure 10 :Lox-catalyzed cooxidation of (A) lycopene and (B) Lycoxanthin from tomato extract in presence of α-tocopherol acetate at different concentrations.

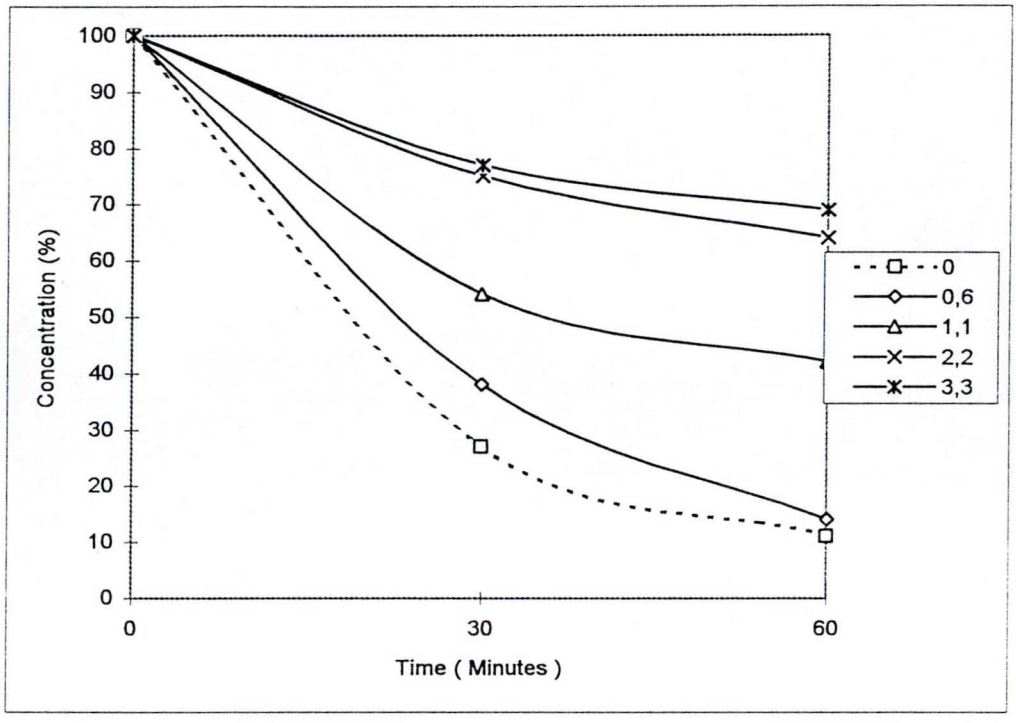


Figure 11 : Lox-catalyzed cooxidation of B-carotene from tomato extract in presence of -tocopherol acetate at different concentrations.

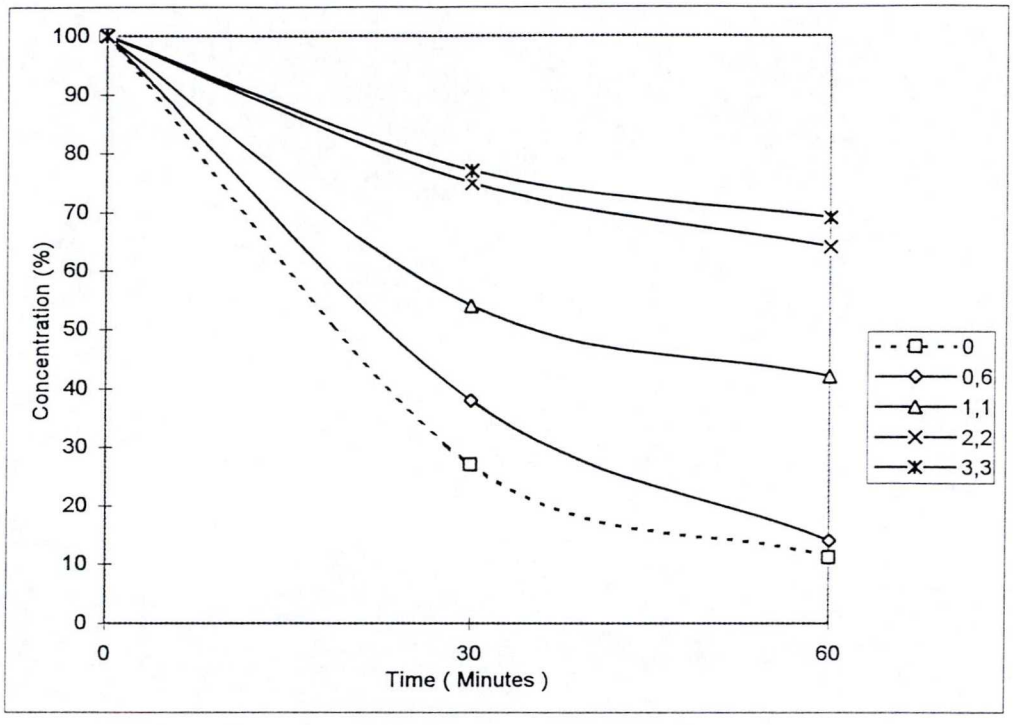


Figure 12 : Lox-catalyzed cooxidation of B-carotene from tomato extract in presence of ∝-tocopherol acetate at different concentrations.

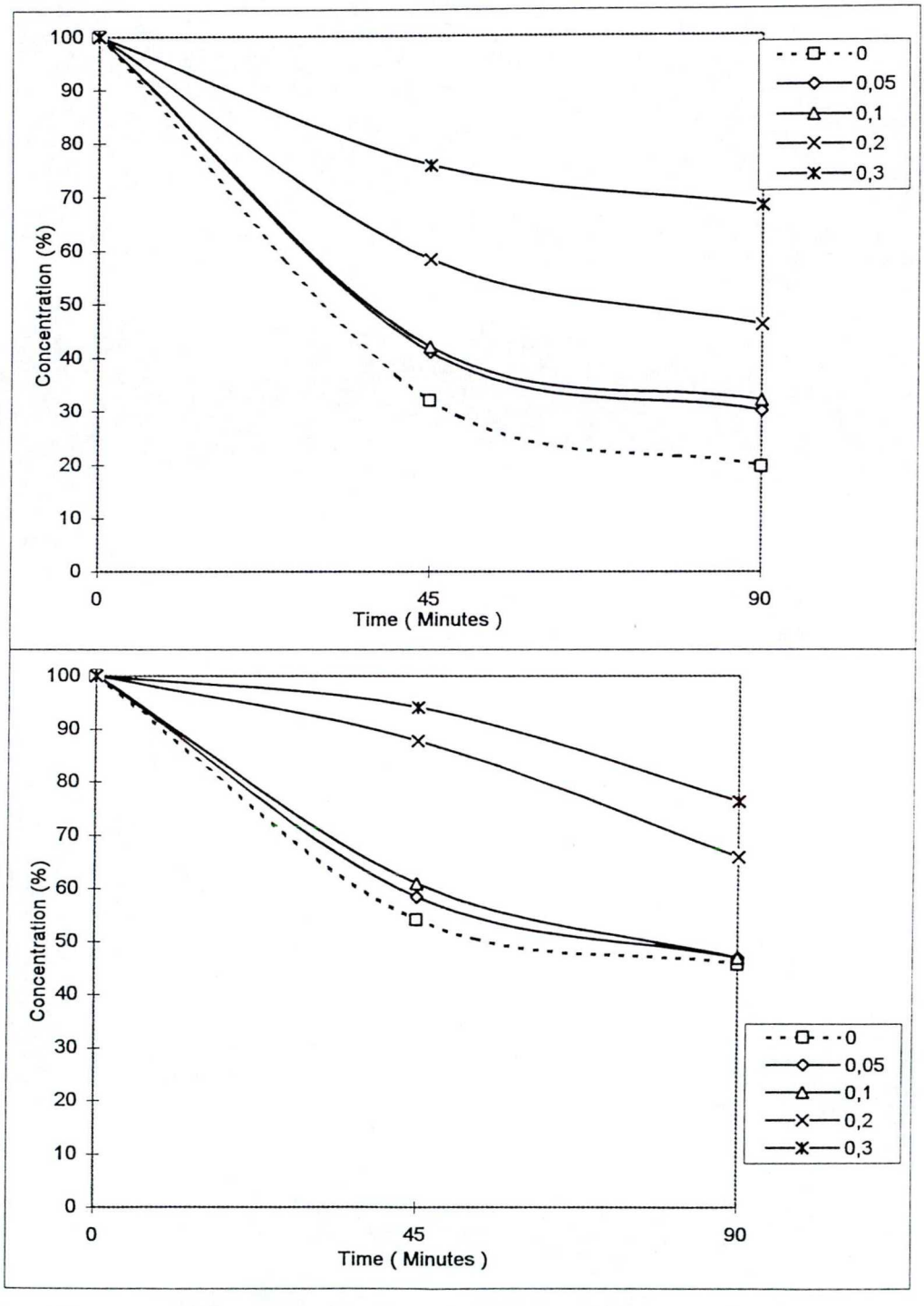


Figure 13 : Lox-catalyzed cooxidation of (A) CME and (B) CDE from spice red pepper in presence of ascorbic acid at different conentrations.

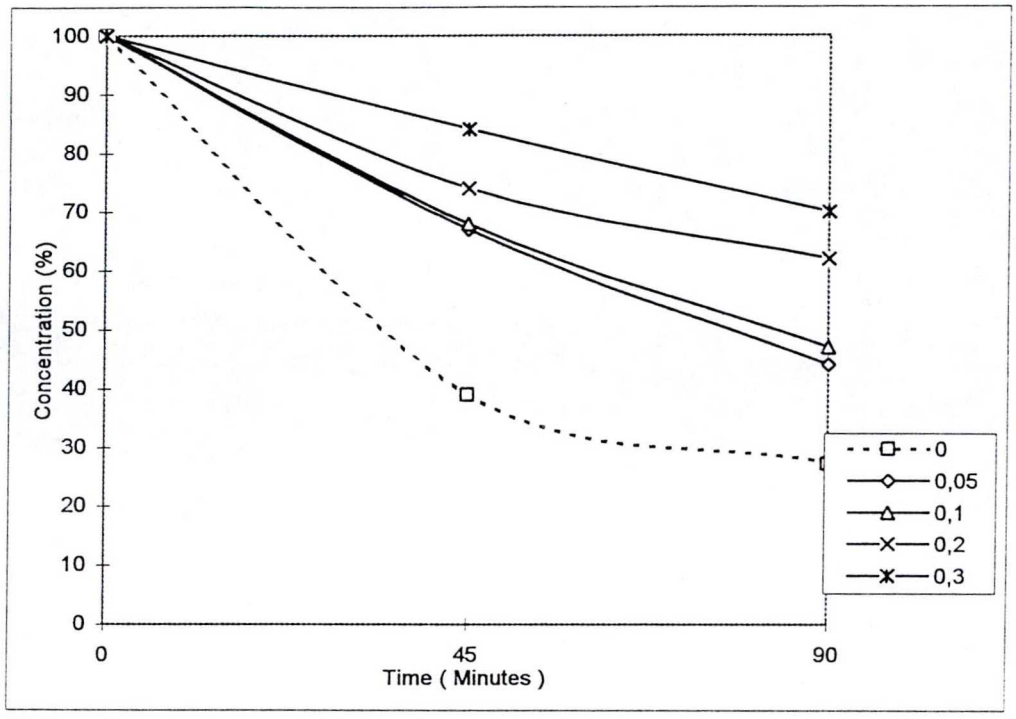


Figure 14 : Lox-catalyzed cooxidation of B-carotene from spice red pepper in presence of ascorbic acid at different concentrations.

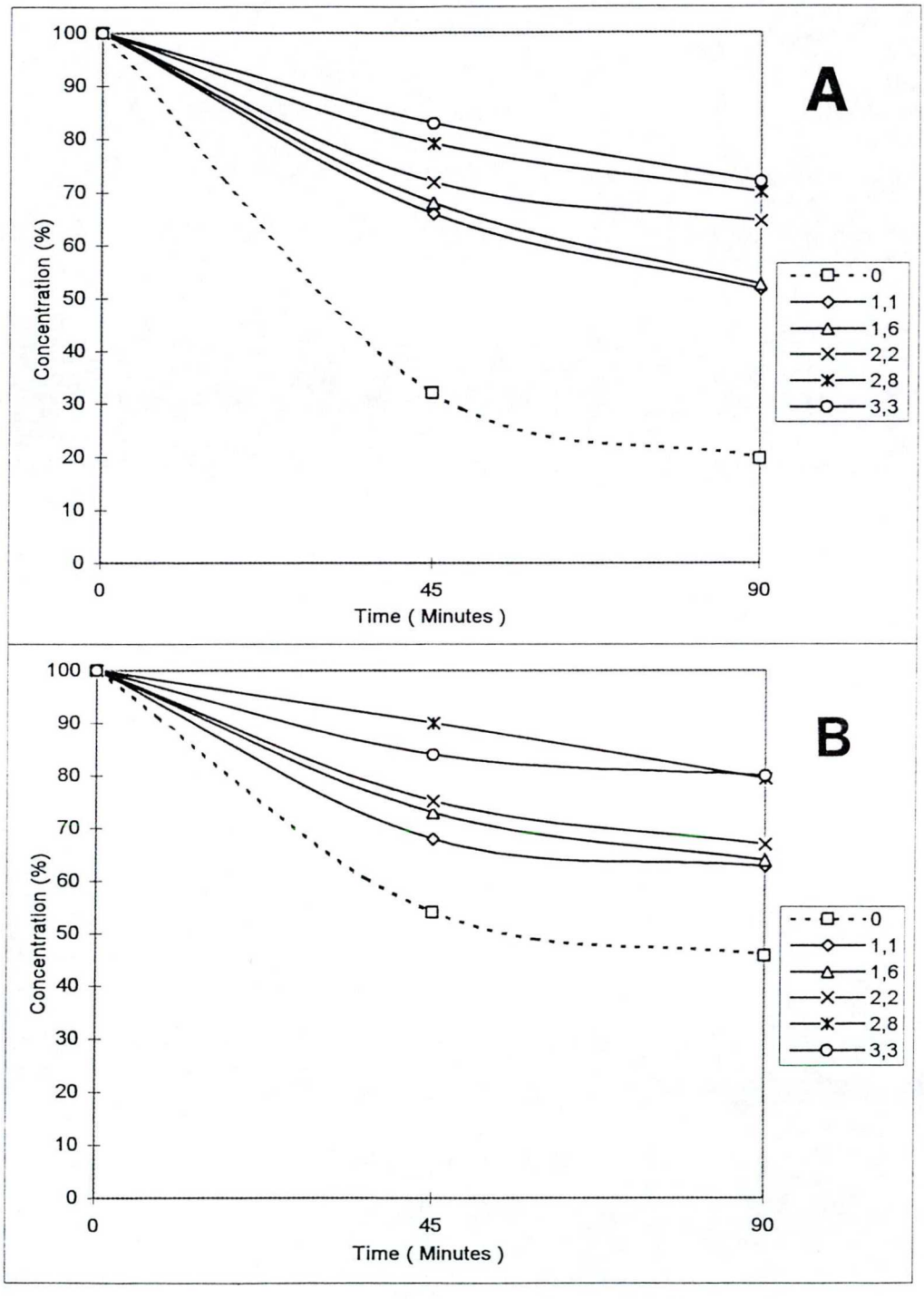


Figure 15 : Lox-catalyzed cooxidation of (A) CME and (B) CDE from spice red pepper in presence of α-tocopherol acetate at different concentrations.

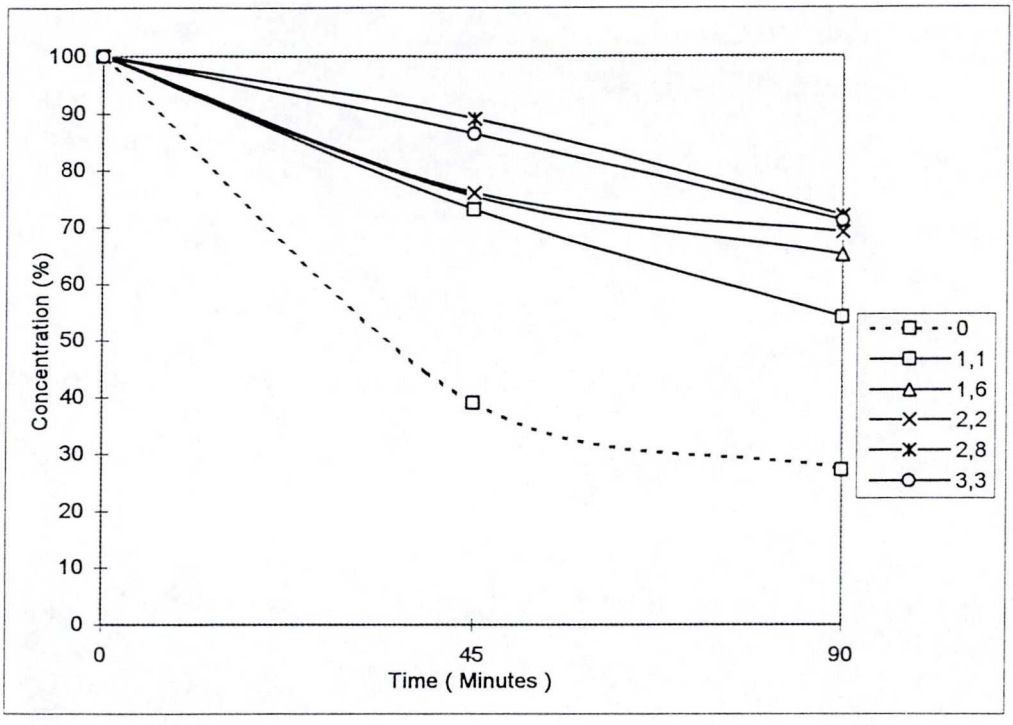


Figure 16 : Lox-catalyzed cooxidation of B-carotene from spice red pepper in presence of α-tocopherol acetate at different concentrations.

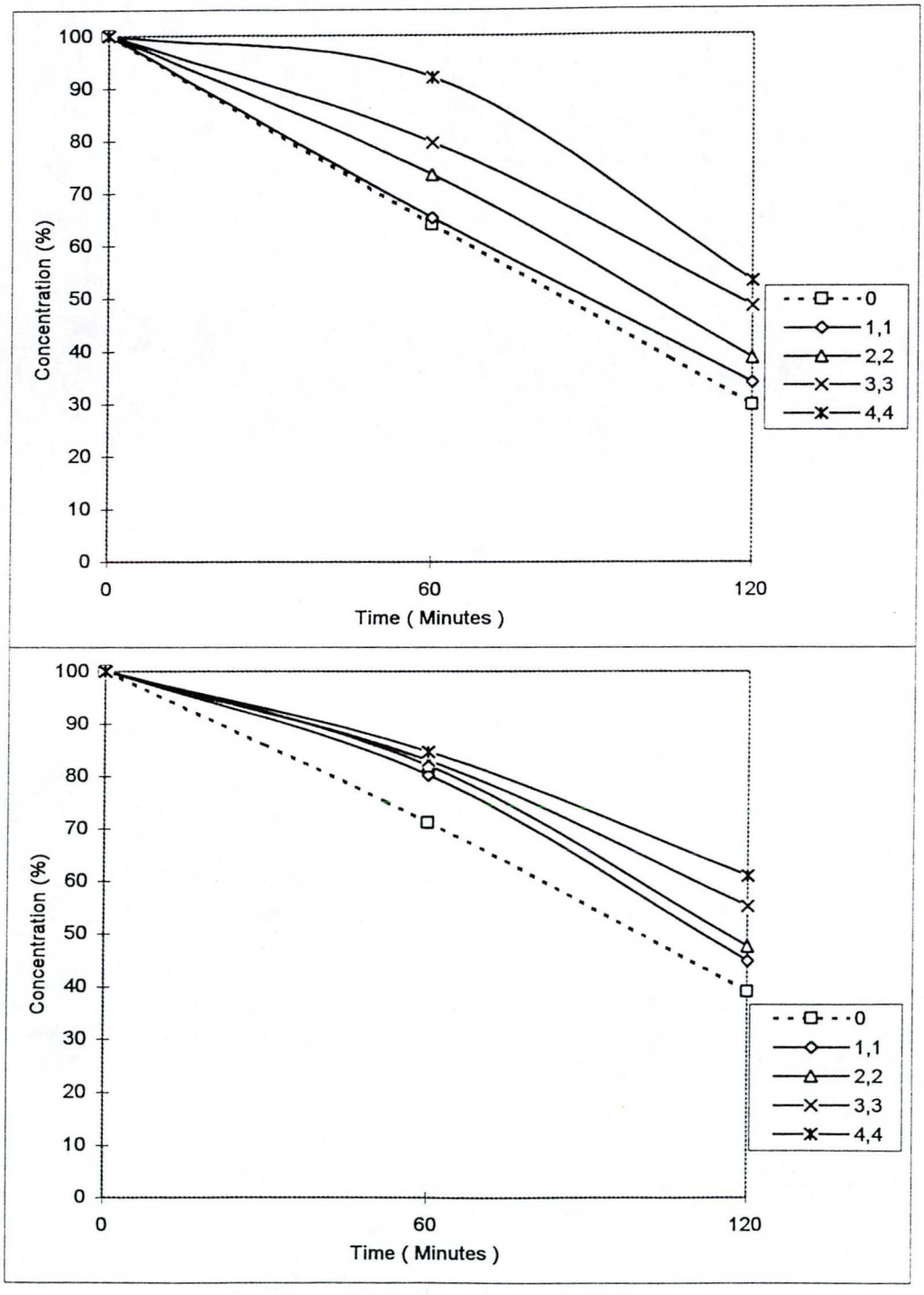


Figure 17 : Photooxidation of (A) chlorophyll a and (B) chlorophyll b from parsely extract in presence of ascorbyl palmitate at different concentrations.

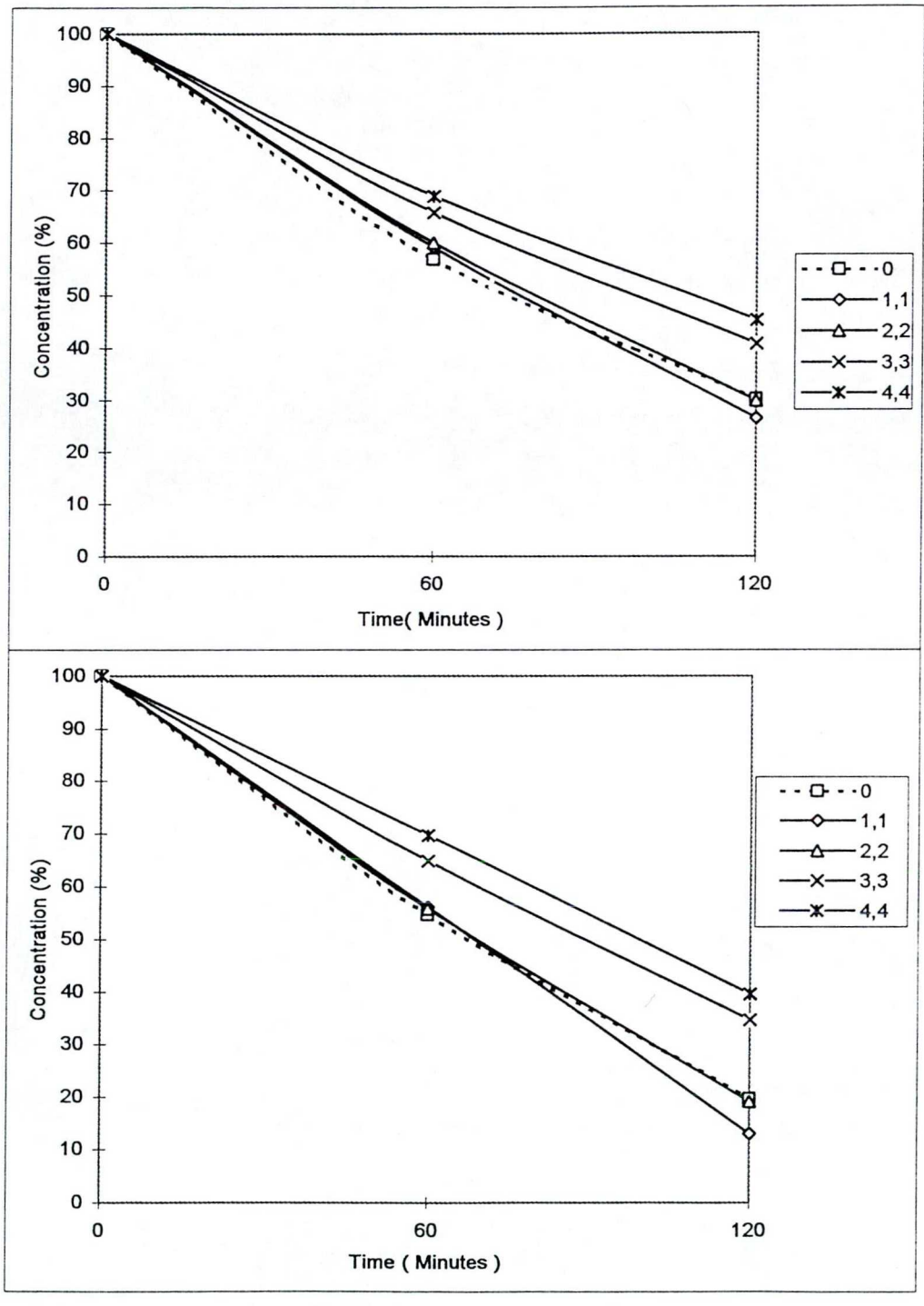


Figure 18 : Photooxidation of (A) lutein and (B) B-carotene from parsely extract in presence of ascorbyl palmitate at different concentrations.

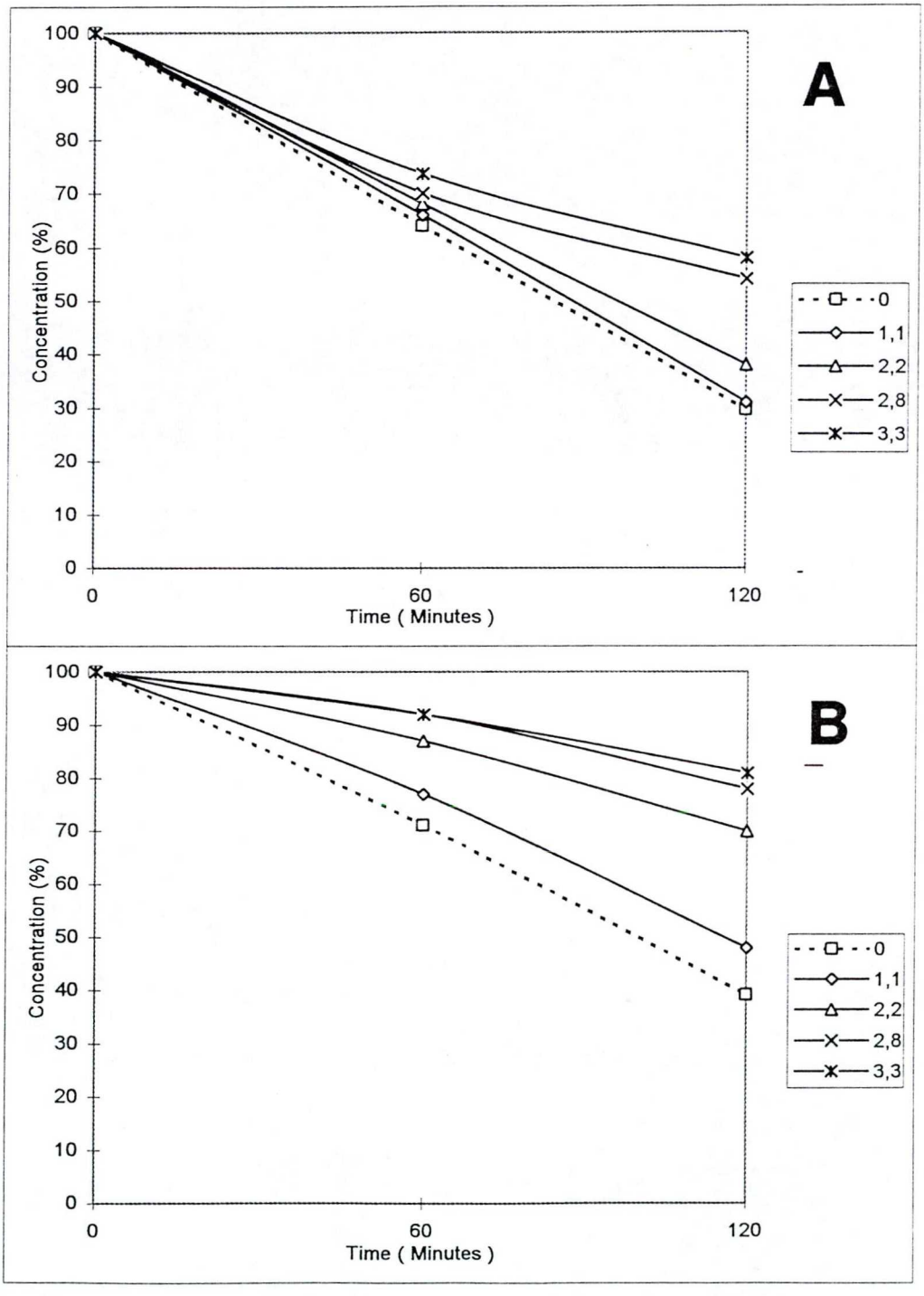


Figure **19** : Photooxidation of (A) chlorophyll a and (B) chlorophyll b from parsely extract in presence of ∝-tocopherol acetate at different concentrations.

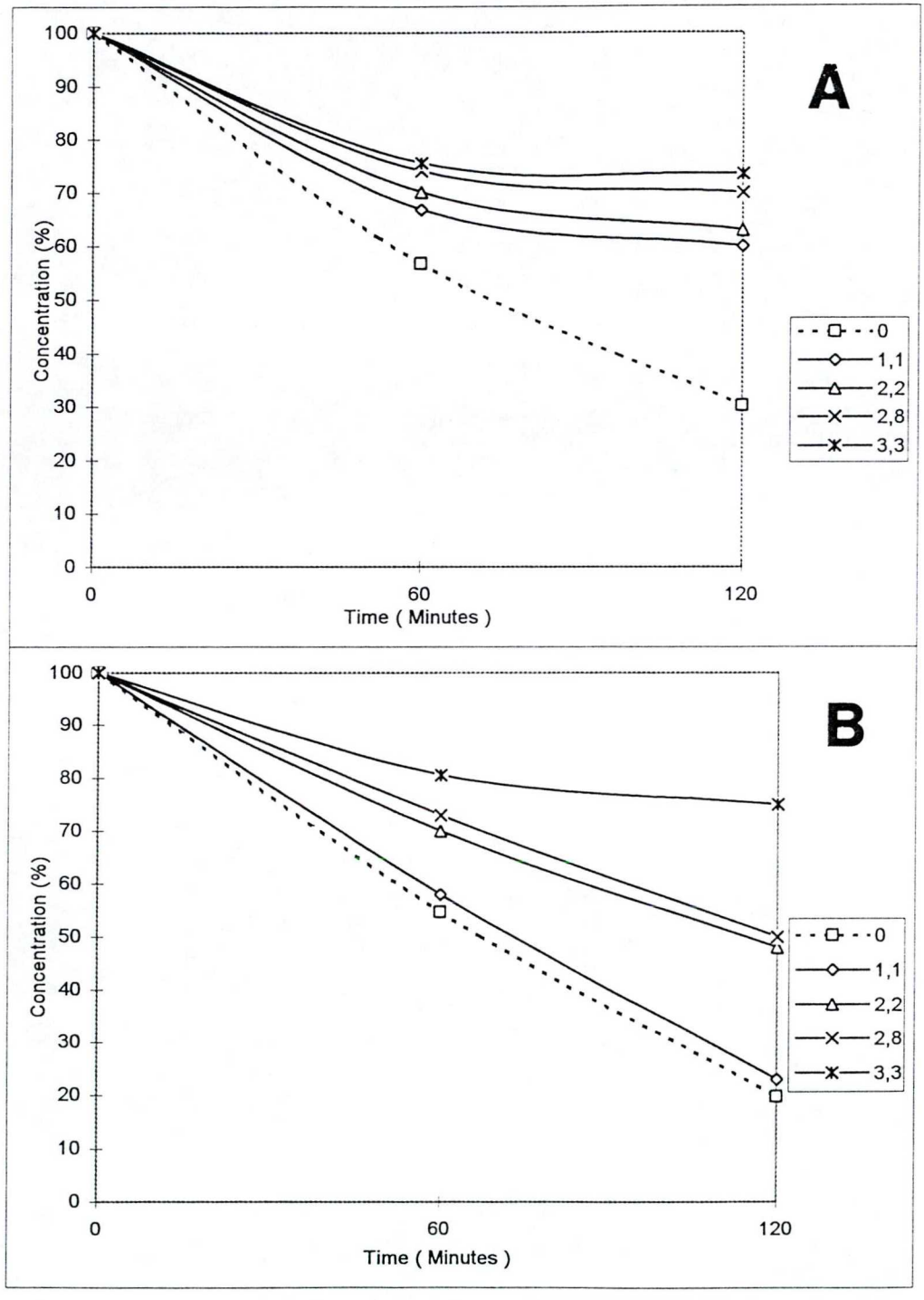


Figure 20 : Photooxidation of (A) lutein and (B) B-carotene from parsely extract in presence of ∝-tocopherol acetate at different concentrations.

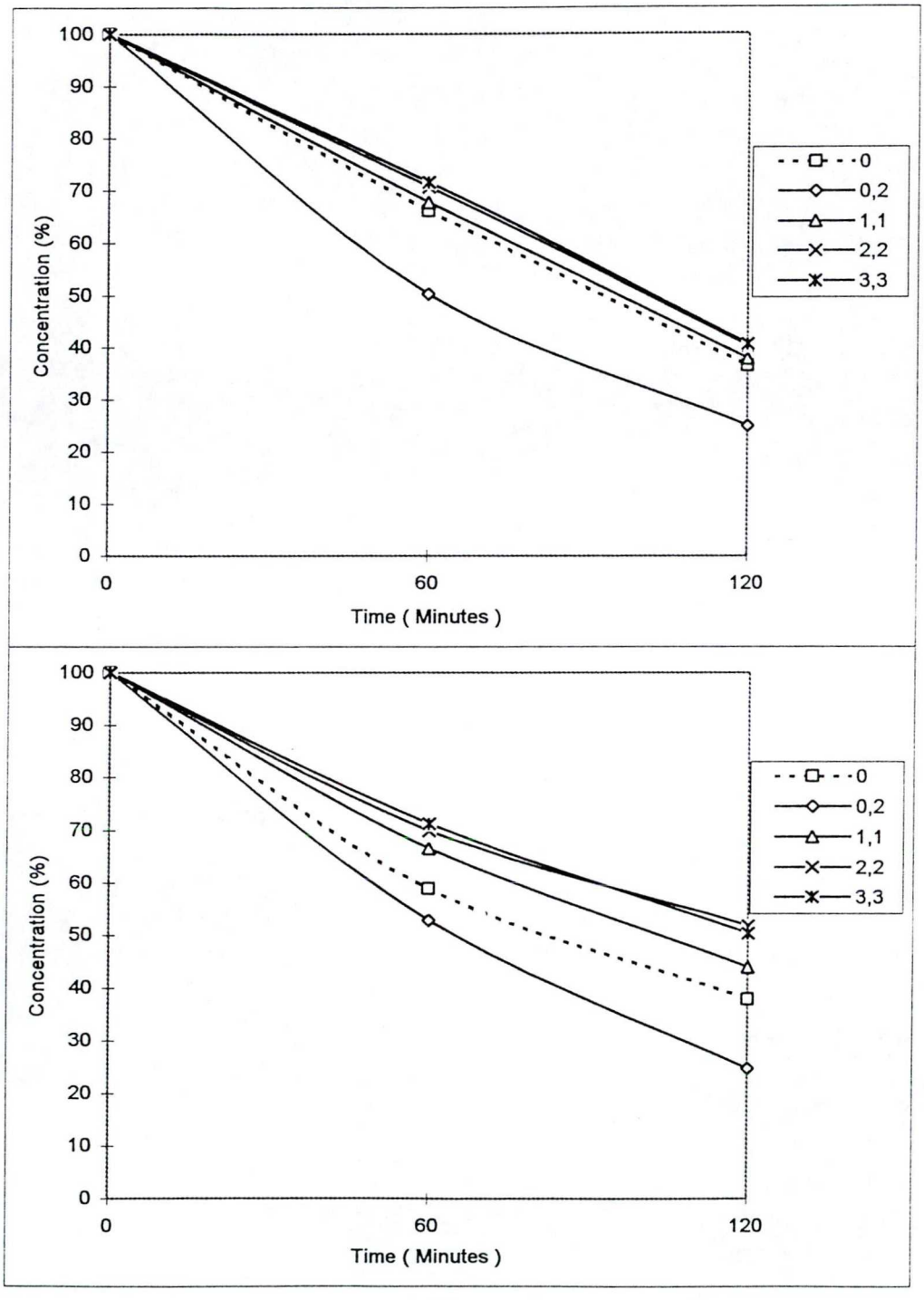


Figure 21 : Photooxidation of (A) lycopene and (B) lycoxanthin from tomato extract in presence of ascorbyl palmitate at different concentrations.

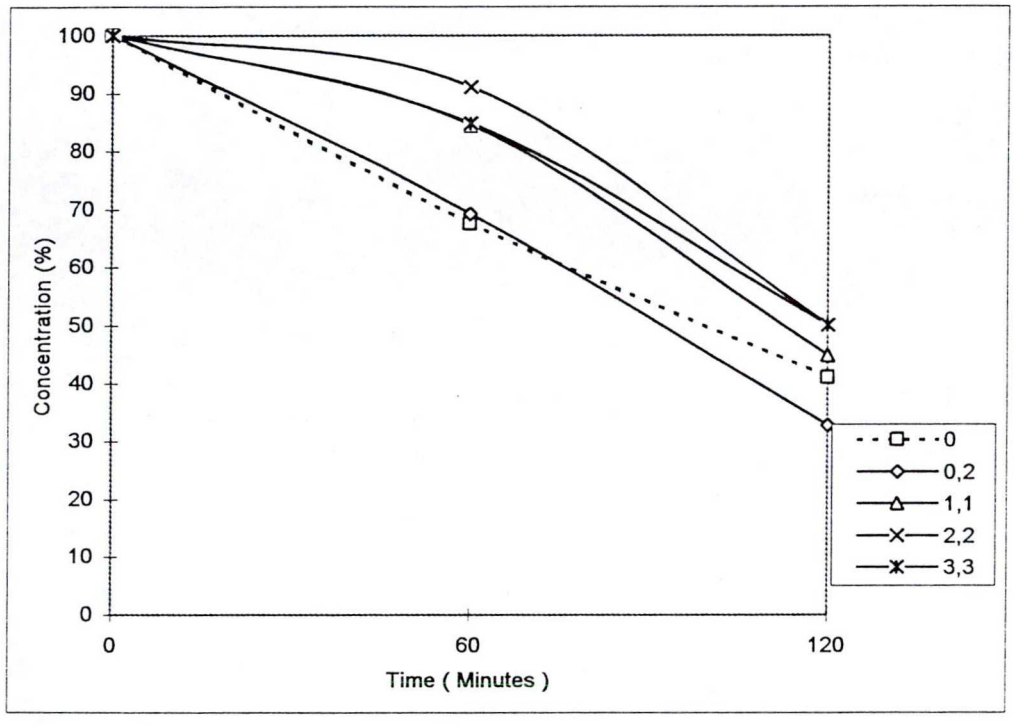


Figure 22 : Photooxidation of B-carotene from tomato extract in presence of ascorbyl palmitate at different concentrations.

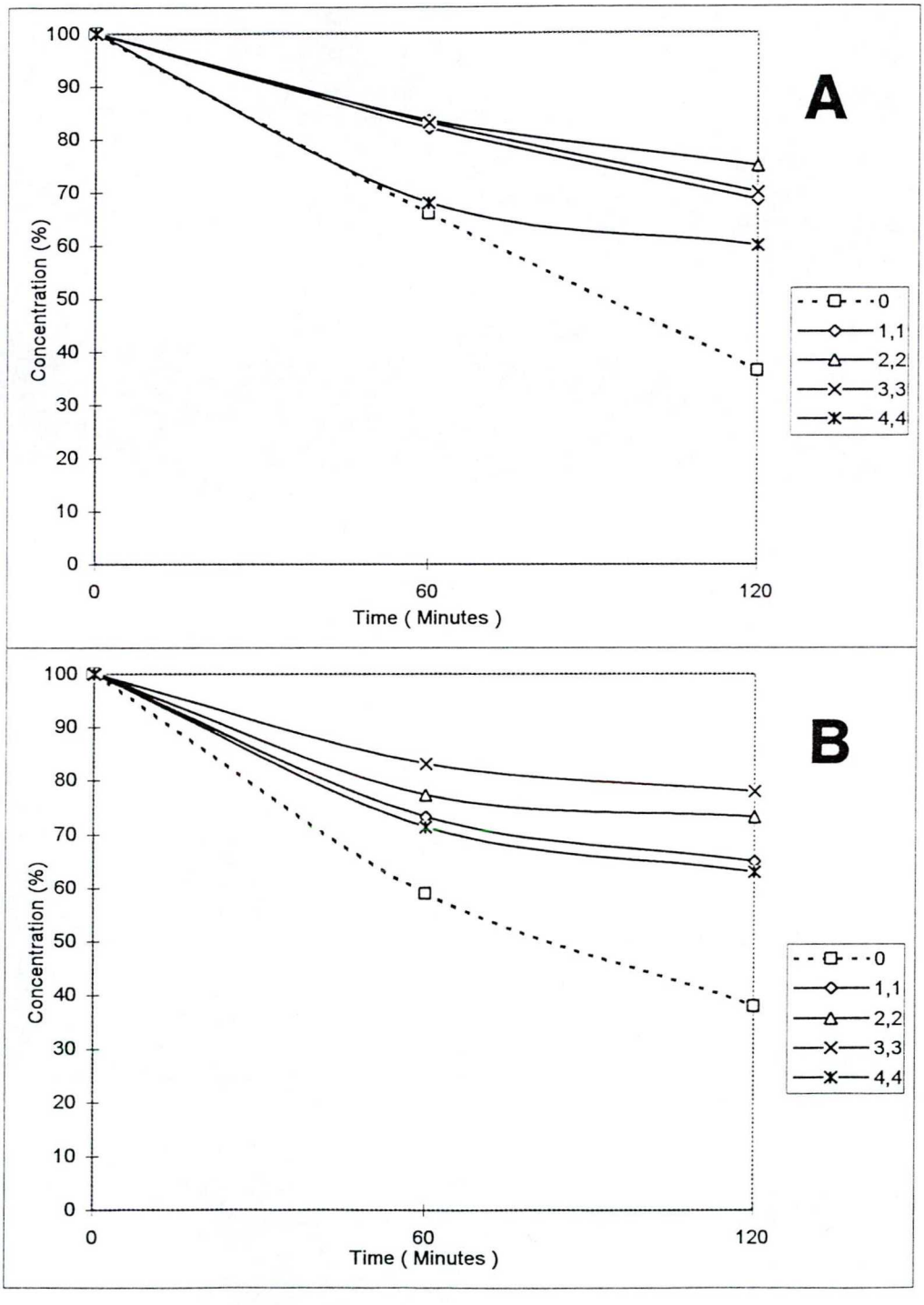


Figure 23 : Photooxidation of (A) lycopene and (B) lycoxanthin from tomato extract in presence of α-tocopherol acetate at different concentrations.

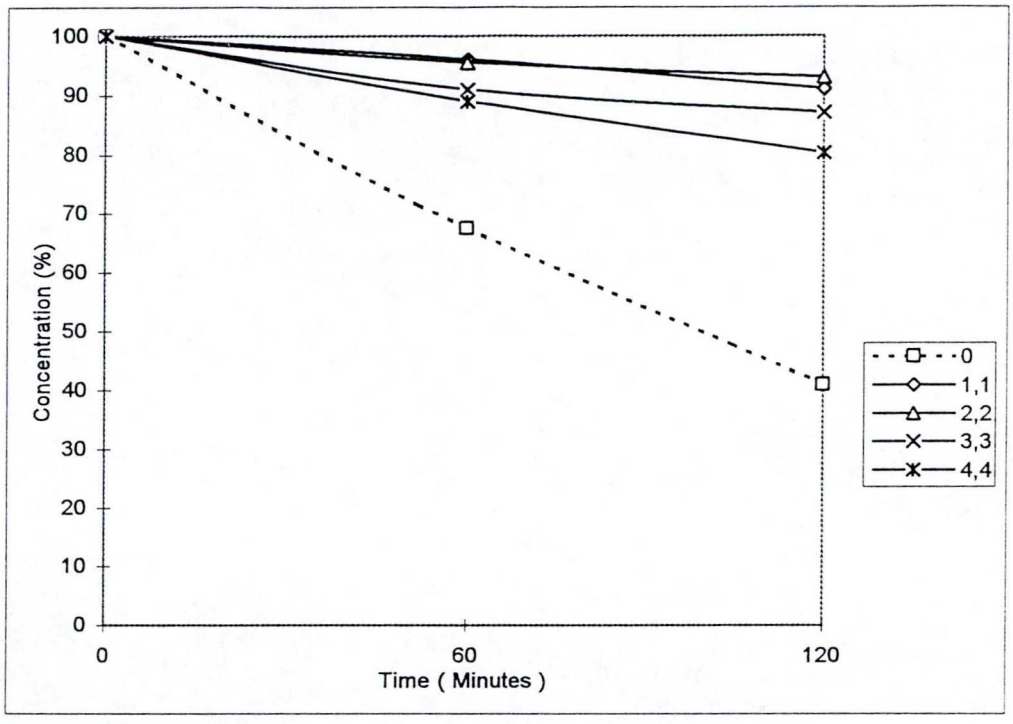


Figure 24 : Photooxidation of B-carotene from tomato extract in presence of∝-tocopherol acetate at different concentrations

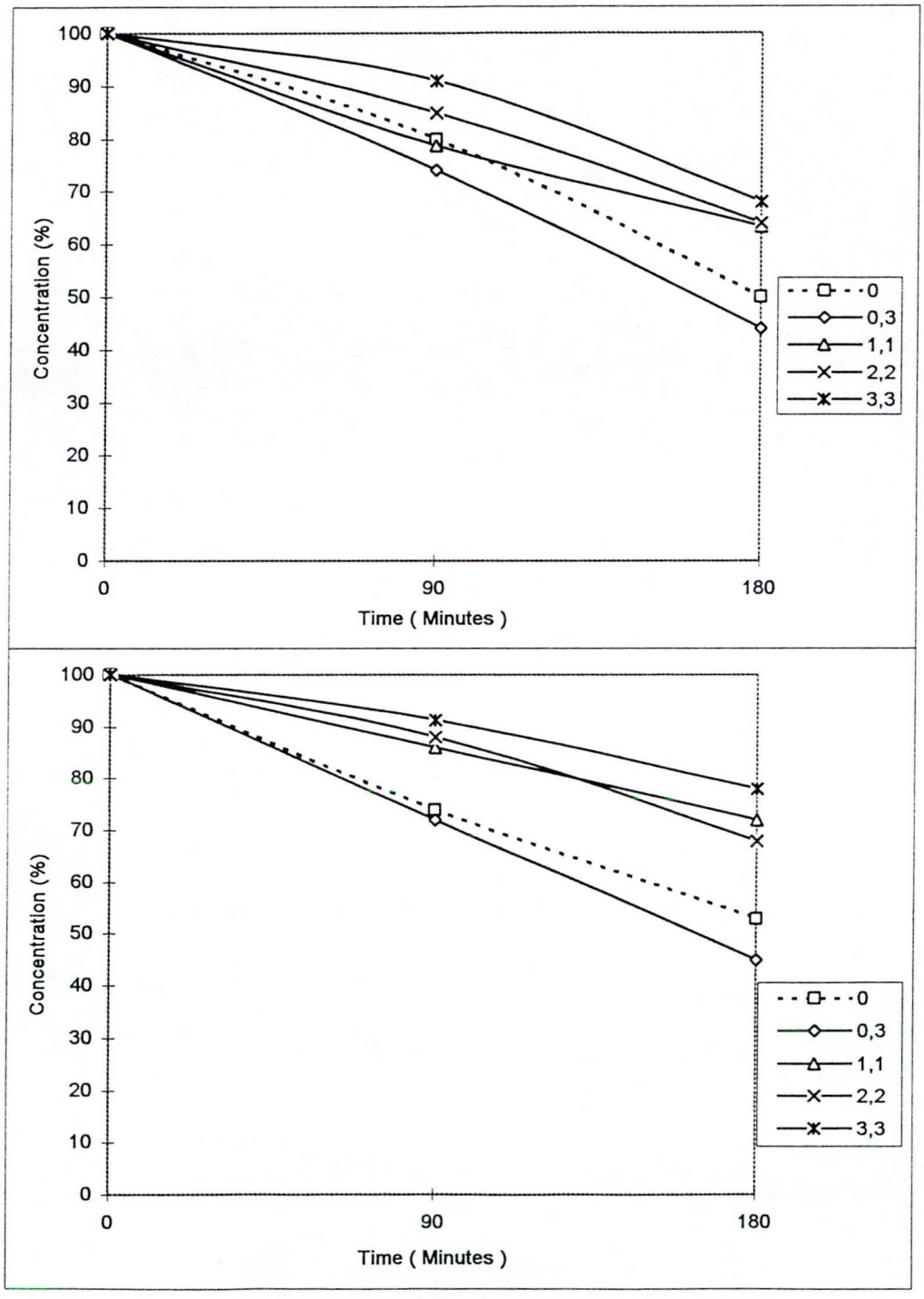


Figure 25 : Photooxidation of (A) CME and (B) CDE from spice red pepper in presence of ascorbyl palmitate at different concentrations.

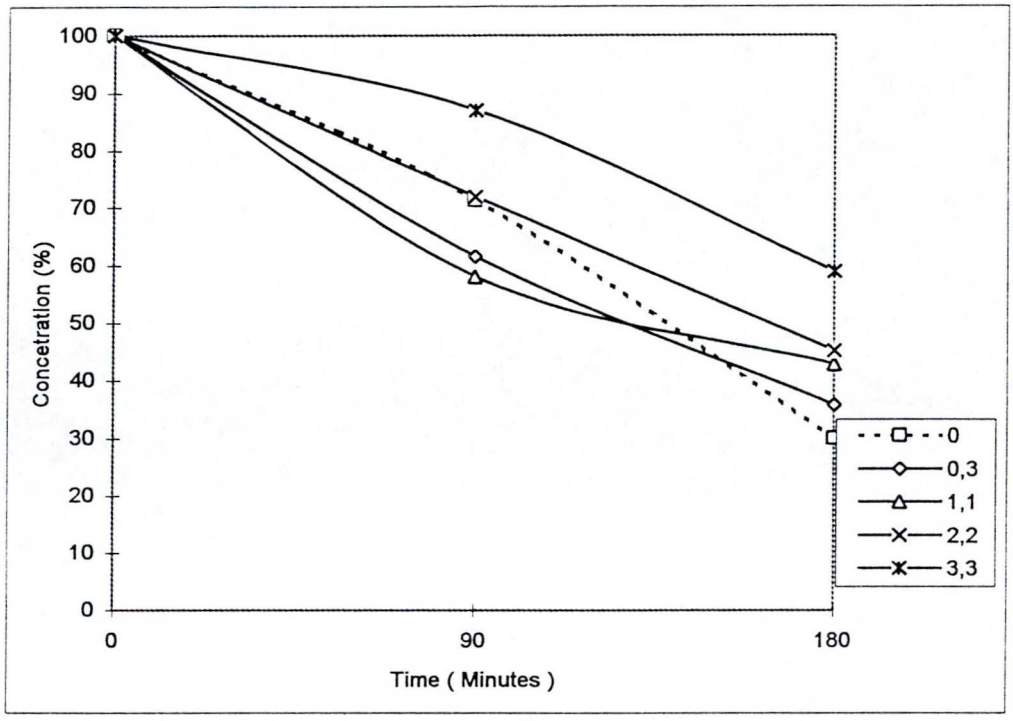


Figure 26 : Photooxidation of B-carotene from spice red pepper in presence of ascorbyl palmitate at different concentrations.

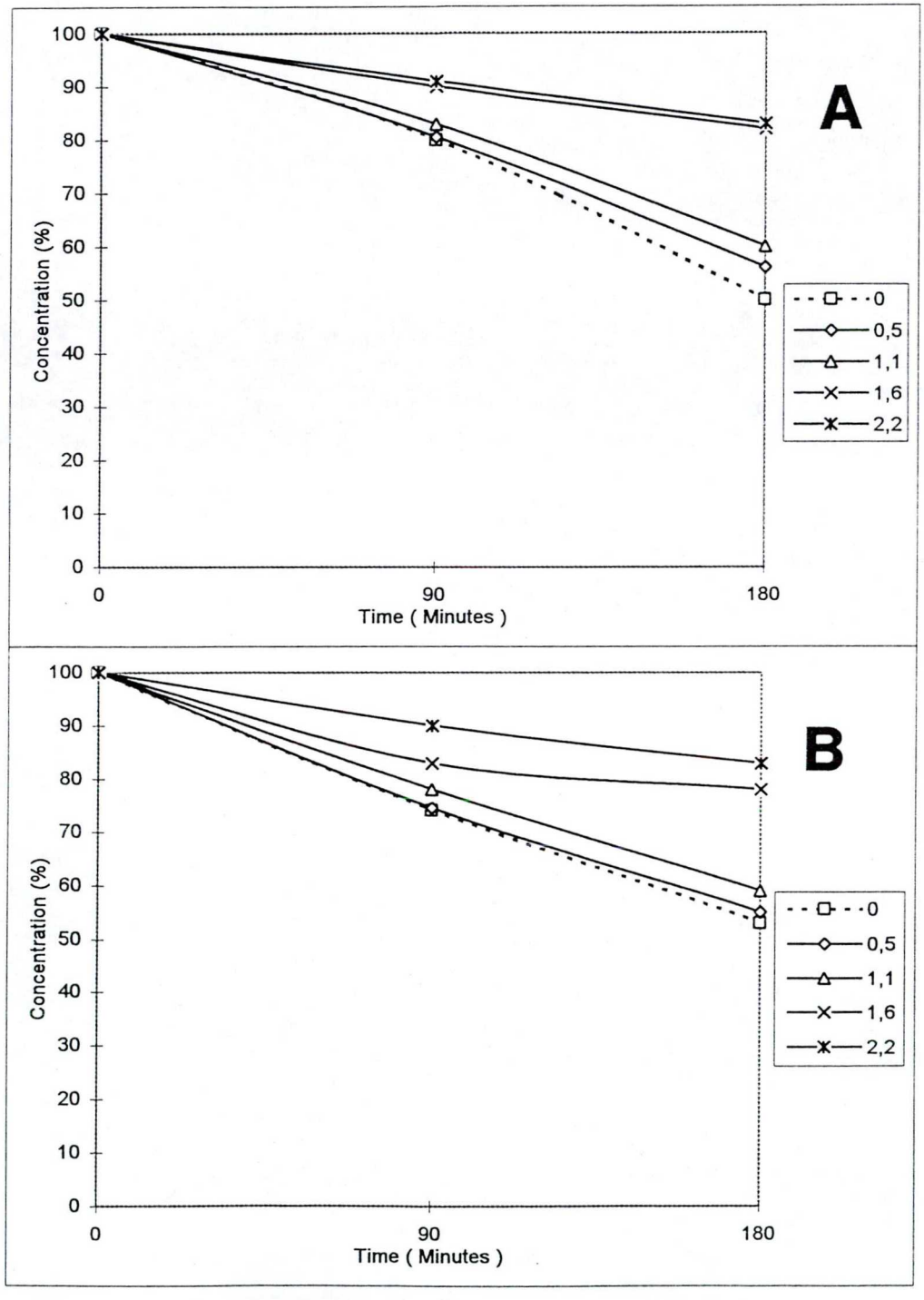


Figure 27 : Photooxidation of (A) CME and (B) CDE from spice red pepper in presence of ∝-tocopherol acetate at different concentrations.

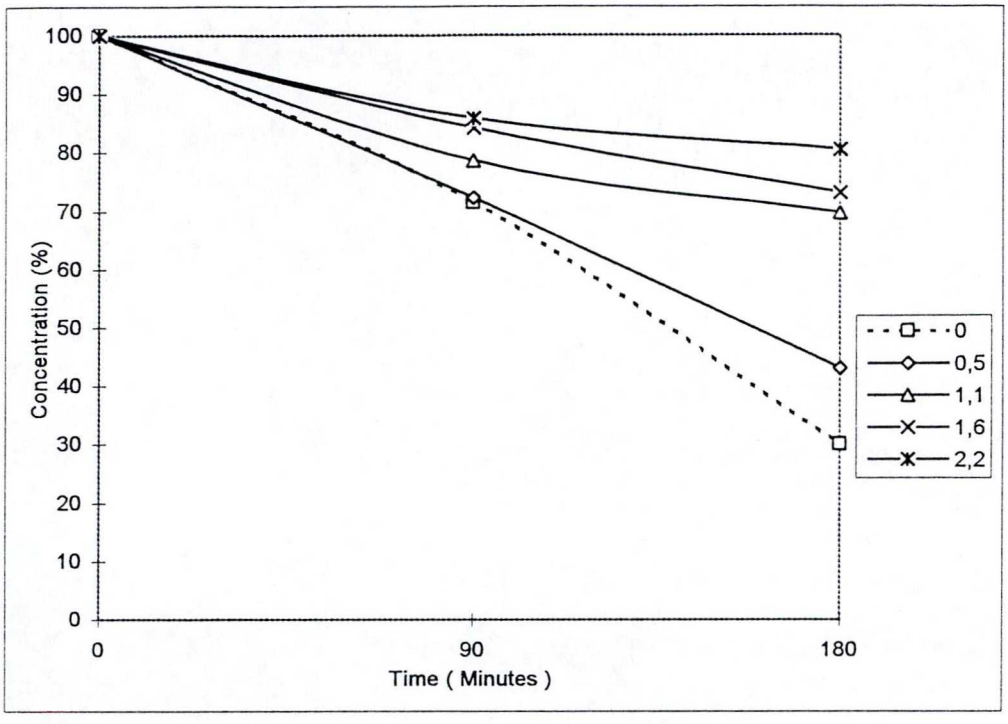


Figure 28 : Photooxidation of B-carotene from spice red pepper in presence of α-tocopherol acetate at different concentrations.