Evaluation of Capsicum as a Source of Natural Antioxidant in Preventing Rancidity in sunflower Oil

Elizabeth Maforimbo

Department of Research and Specialist Services,
Chemistry and Soils Research Institute,
P.O. Box CY550, Causeway, Harare, Zimbabwe

Abstract

Solvent extraction to obtain oleoresins of flavonoid and carotenoid extracts was done from 200 grams of commercial paprika powder. Petroleum ether was used to defat the sample. Methanol and n-butanol were used in turn to extract the polar flavonoids, while chilled acetone and petroleum ether were used in turn for the extraction of nonpolar carotenoids.

The flavonoid yield was 5.92grams while that for the carotenoids was 39.15 grams per 100g of the powder. These residues were tested on sunflower oil to evaluate their antioxidant effects. The accelerated methods used for oxidation were the Automated Rancimat, Oven heating and UV light at 254 nm. The oxidation of sunflower oil was measured using changes in peroxide values and the UV absorbances (Conjugated diene) at 232 nm, $E^{1\%}_{1cm}$.

The extracts tested possess antioxidant effect and this phenomenon was enhanced by increasing their concentration, since they were impure. The major components of paprika (b-carotene and capsanthin) were also tested on the oil as pure standards while Butylenehydroxytoluene (BHT) was used as a standard antioxidant.

Results showed that carotenoids seemed to be effective in retarding oil peroxidation under photoxidation, while showing pro-oxidant activity under oven heating autoxidation, and showing slight antioxidant activity under Rancimat autoxidotion tests. The flavonoids were effective in retarding oil oxidation under both photoxidation and thermal autoxidation tests, with the column separated fractions 3 and 4 conferring better antioxidant activity under the Rancimat test. These column separated fractions, were identified by the UV Spectra as 3-hydroxyflavanones or dihydroflavonols.

Key words: Paprika, Carotenoids, Flavonoids, natural antioxidants.

Introduction

The aims of this work were to isolate and identify natural antioxidants from a capsicum fruit variety and to establish the mechanism of action of any antioxidants identified. The capsicum fruits, paprika and pepper are largely cultivated in Zimbabwe for flavour enhancement. Therefore, an establishment of antioxidants from these fruits would benefit the consumer.

Natural antioxidants are primarily plant polyphenolic compounds that occur in all parts of the plant. Plant tissues are the main biological systems that synthesise a-tocopherol, ascorbic acid and carotenoids. They are also rich in a wide variety of phenolic compounds. The flavonoids and other plant phenolics have been reported to have multiple biological effects such as antioxidant activity, (Kanner, and Frankel 1994.) The use of Tocopherol as a natural antioxidant is not superior to artificial antioxidants such as Butylenehydroxytoluene (BHT) and Butylenehydroxyanisole (BHA). Consequently, tocopherols have been used with synergists. Therefore, the development of an "effective natural Antioxidant" or synergist is highly anticipated. (Fish., 1993.)

Synthetic antioxidants may act as mutagenic and/or carcinogenic agents, (Ramanathan and Das 1993). Questions about the safety of synthetic antioxidants are increasingly plaguing the industry, forcing fats and oils product manufacturers to rethink their use of these products. One result has been the decrease in their use with renewed interest in Naturally derived Antioxidants, (Fioriti and Sims, 1990).

There is therefore growing consumer preference for "natural antioxidants". The trend towards the use of more unsaturated fats means more antioxidants to counteract the oxidation processes are needed. Jorge *et al*, reported tocopherol, ascorbic acid, and b-carotene as compounds effective against Cancer, heart disease and cataracts.

Photoxidation of vegetable oils is a major concern of the food industry, because these oils contain natural photosensitizers and are commercially sold under light. Not only are these vegetable oils susceptible to oxidation due to high concentration of linoleic acid but they contain chlorophyll and their decomposition products which are potential photosensitizers thus, generating singlet oxygen in the presence of light and triplet oxygen. The singlet oxygen formed reacts with double bonds of unsaturated fatty acids to form the initial hydroperoxides which later break down and form radicals to initiate autoxidation, (Dondeena and David, 1992).

Carotenoids are highly oxidizable and this provides a protective action against radiation (light) damage of the medium in which they are dissolved. This oxidation is a free radical process and causes their ultimate destruction, and in this way, a bleaching of the colour. Radical quenchers like carotenoids act by protecting crude fats and oils once they have been produced only against light induced photoxidation by transforming the absorbed radiation energy into heat (Hoffman, 1989)

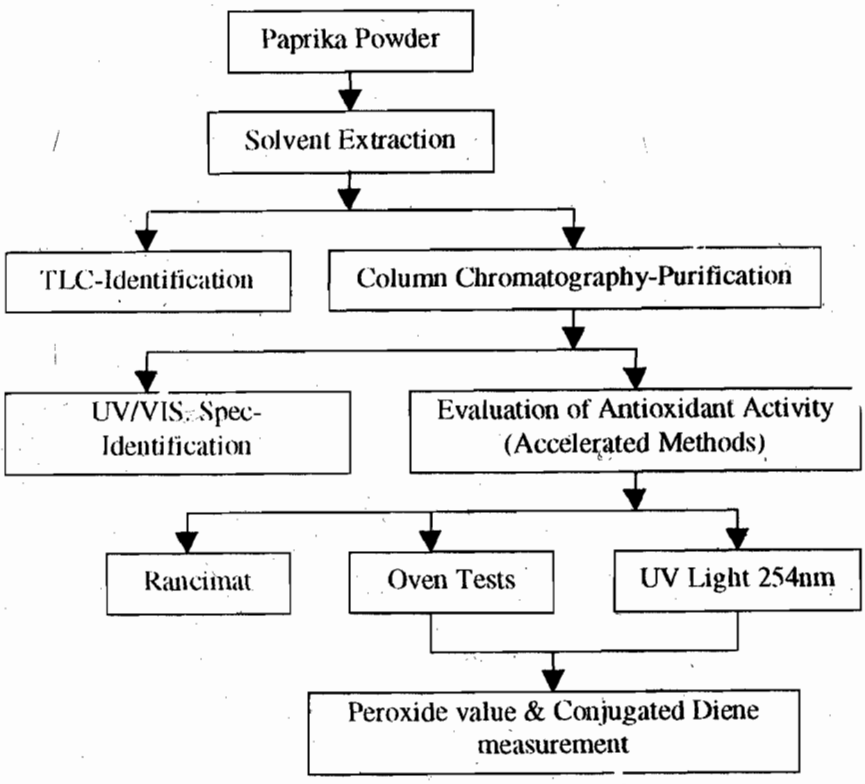
Paprika, *(Capsicum annum L)* belongs to the night shade family Solanaceae (Rosalita *et al.*, 1969). The Keto-Carotenoids, capsanthin and capsorubin, comprise 70-80% of the total carotenoids of paprika and contribute to its unique red colour. Paprika is also high in b-carotene and xanthophyll pigments.

Rancidity in fats and oils is the result of autoxidation of the unsaturated fatty acids. It is one of the major changes that occur in lipids and lipid based foods during processing, distribution and final preparation. The oxidation of lipids initiates other changes in the food and this eventually affects the food's nutritional and organoleptic qualities.

Autoxidation of polyunsaturated lipids involves a free radical chain reaction that is most frequently initiated by exposure of lipids to light, ionisation radiation, metal ions and metalloprotein catalysts. It starts when the first hydroperoxides decompose to alkoxy and hydroperoxy radicals. These radicals abstract a H·. from vulnerable sites in monoenoic and polyenoic (eg, linoleic) fatty acid residues in the fats/oils, and foods containing fats/oils (Coultate, 1996).

The mechanism of phenolic antioxidants including flavonoids is by donation of a hydrogen atom to the lipid radicals, reactions (1 & 2). Reactions (3 & 4) compete with the propagation stages;

1)ROO· + AH ROOH +A·
2)RO· + AH ROH + A·
3)ROO· + A· ROOA
4)RO· + A· ROA
5)ROO· + RH ROOH + R·



Materials and Methods

The methods for extraction of flavonoids from paprika, Column Chromatography for separation of crude fractions and UV/VIS Spectroscopy for identification of compounds were adapted and modified from Tsaknis, (1996) (*see chart above*). The methods for carotenoid extraction, Thin Layer Chromatography for separation of crude fractions and UV/VIS Spectroscopy for identification of compounds were adopted and modified from Buckle and Rahman, (1979).

Accelerated methods to induce autoxidation and photoxidation of the oils and to evaluate antioxidant activity were adapted from Tsaknis, (1996).

The accelerated methods for oil oxidation:

Rancimat: This is based on conductometric determination of the volatile degradation products of oil oxidation, and features automatic plotting of the conductivity against time. The time taken for acid generation before the conductivity of the water increases rapidly will indicate the induction period.

Oven method: Fat/oil sample is exposed to elevated temperatures in a temperature controlled oven to increase the rate of oil oxidation and thus peroxide value is increased.

Ultra-violet method: Oil oxidation is accelerated by UV light (260nm) without elevating temperatures at which volatile compounds might evaporate. Petri dishes of oil are placed in a thermostat tank maintained at 50±5 °C under UV light.

The evaluation of oil oxidation

Peroxide Values (PV) measures the formation of intermediate hydroperoxides in milliequivalents of active oxygen per kilogram of sample. It is determined by the volumetric determination of the iodine liberated from potassium iodide by oxidation with peroxides at room temperature in chloroform-acetic medium.

The increase in the UV absorbance at 232nm due to conjugated diene formation from the unsaturated fats is an index of progressive staling of the oil, (St Angelo and Allen., 1992).

Results

Capsanthin, b-carotene and lutein were positively identified from the petroleum ether extract using UV/VIS Spectroscopy and Thin Layer

Fig 1. Antioxidant activity of flavonoid extracts from paprika powder and BHT in sunflower oil with the Oven heat at 65°C

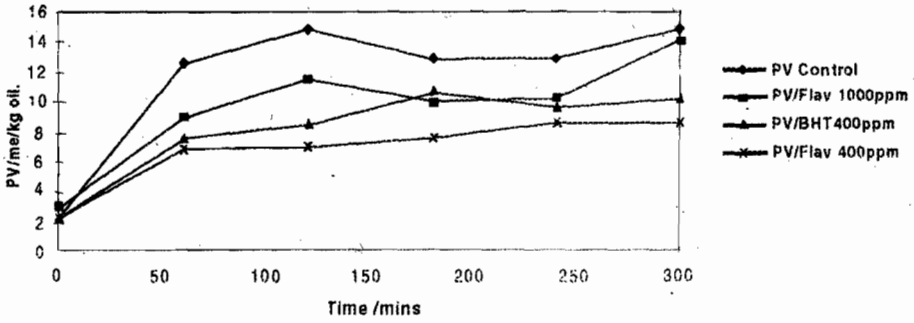


Fig 2. Antioxidant activity of pure beta carotene std and carotenoid extr of paprika powder in sunflower oil using UV (254nm)

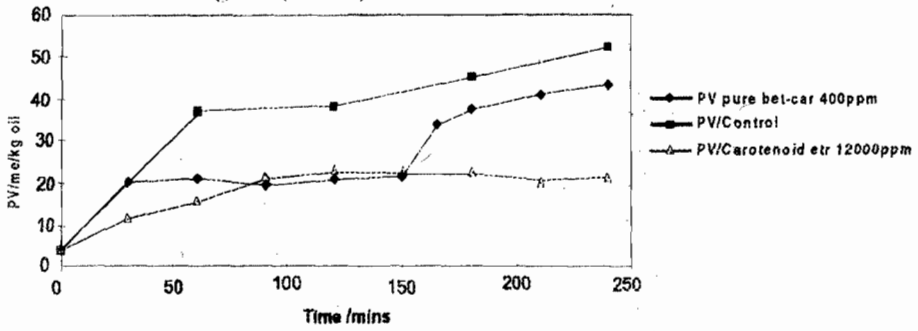


Fig 3. Antioxidant activity of flavonoid and carotenoid extracts from paprika powder and standards on sunflower oil with UV (254nm) accelerated oxidation

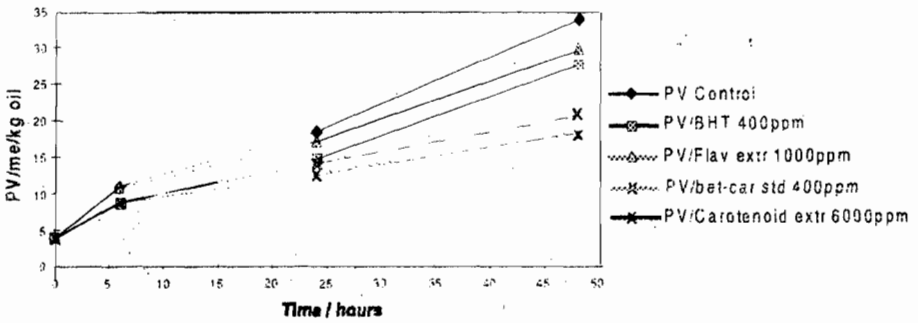
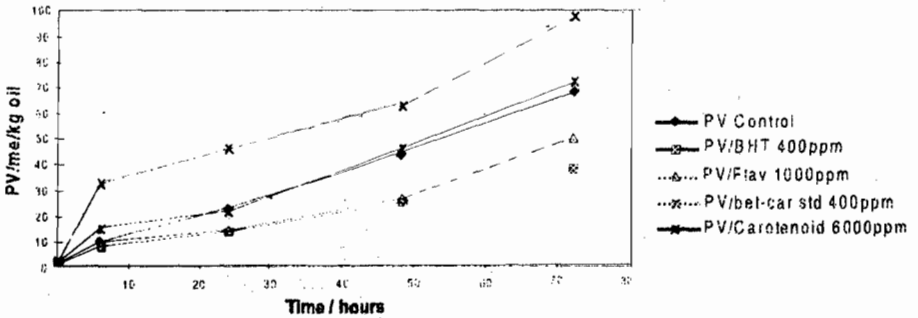


Fig 4. Antioxidant activity of flavonoid and carotenoid extracts from paprika powder and standards on sunflower oil with Oven heat (55°C) accelerated oxidation



Chromatography. From the n-butanol extract, the polar flavonols; dihydroflavonols and flovonones (3-hydroxyflovonols), were positively identified.

Antioxidant Activity of Extracts

Under the Rancimat Autoxidation tests, beta -carotene standard at 400 ppm, BHT at 490 ppm, and crude carotene at 6000 ppm (an equivalence of 390 ppm beta-carotene), gave protection factors of 1.2; 1.33; and 1.05 respectively.

The column-separated fraction 3 from the crude flavonoid extract gave the highest antioxidant activity at 2% concentration, giving a protection factor of 1.81. This fraction 3 had the highest concentration of dihydroflavonols, (3-hydroxyflovonones), which seemed to confer the antiperoxidative properties owing to their structure.

Protection factor (PF) or the antioxidant index expresses the ratio of the induction periods for the stabilised and unstabilised oil. Thus a P.F. greater than 1 indicates inhibition of lipid oxidation. The higher the PF, the better the antioxidant activity (Tsaknis, 1996).

Oven tests results; effect of flavonoids: For the Oven accelerated oxidation of the oil, at a temperature of 65°C and up to 5 hours, the peroxide values (PV) for the flavonoid extract at 400 ppm in oil were the lowest, (highest protection to oil oxidation) followed by BHT at 400 ppm and the crude flavonoid at 1000 ppm/oil (**fig. 1**).

UV accelerated oxidation- effect of Carotenoids: With UV light at 254 nm up to 4 hours, crude carotenoid extract at 12000 ppm (which is the equivalence of 780 ppm of pure b-carotene), and the standard b-carotene at 400 ppm retarded the rate of oil peroxidation (**fig 2**), with the control oil showing a higher rate of peroxidation.

During another experiment with UV light at 254 nm, up to 48 hours, crude carotenoid extract at 6000 ppm (equivalence of 390 ppm pure b-carotene), reduced the rate lipid peroxidation most effectively, followed by pure beta-carotene at 400 ppm, BHT at 400 ppm and flavonoid extract at 1000 ppm respectively. This is illustrated in **fig 3**.

Carotenoid extract at 6000 ppm and beta-carotene standard at 400 ppm accelerated the rate of oil oxidation as shown in **fig 4**. BHT at 400 ppm and flavonoid extract at 1000 ppm effectively slowed down the rate of oil oxidation.

The control oil (with no protection), had the highest UV absorbance at 232nm followed by BHT and b-carotene treated oils. The oil treated with carotenoid extract at 6000 ppm had the lowest UV absorbance, ie highest protected oil. Flavonoid extract at 1000 ppm also protected the oil to a lesser extent than the carotenoid extract at 6000ppm (**fig. 5**)

Fig 5. UV Abs at 232nm, E after 48 hours of UV light at 254nm

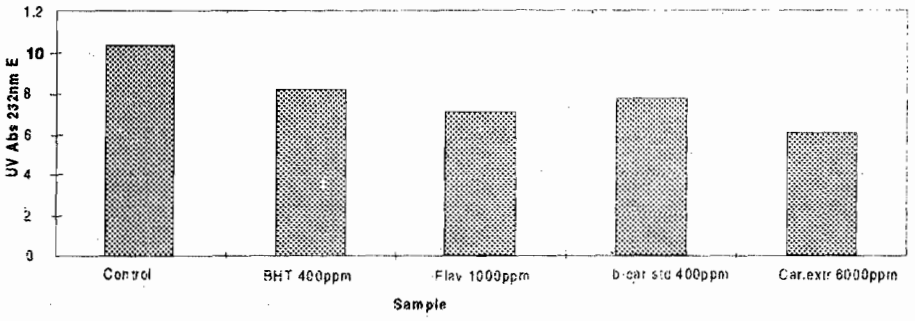
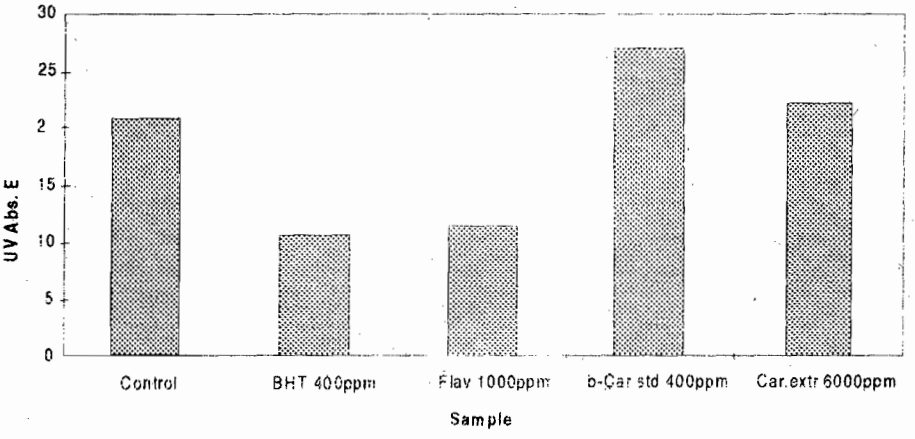


Fig 6. UV Absorbances 232nm (conj.diene) for extracts and standards on sunflower oil after 48 hrs of oven heat at 55°C.



The beta-carotene standard at 400 ppm showed pro-oxidant properties as can be compared to the control oil with no protection. Carotenoid extract at 6000 ppm again showed pro-oxidant properties. BHT at 400 ppm and flavonoid extract at 1000 ppm had lower UV absorbances showing highest protection to oil oxidation (**fig. 6**).

Discussion

During thermal autoxidation in the dark, (Oven heating and Rancimat), radical autoxidation occurs when hydroperoxides and peroxides are breaking down and generating more free radicals to maintain the chain reaction. The BHT and the flavonoids therefore seem to be more efficient in that they are effective hydrogen donors, i.e. blocking up the free radicals formed during the propagation stage. These phenolic antioxidants, are excellent hydrogen or electron donors and their intermediates are stabilised by resonance (**fig 4**).

On the other hand, the Carotenoids, ie the b-carotene and the crude carotenoid extract could not protect the oil during thermal autoxidation, which occurred in the absence of light. b-carotene seems not to have any effect on this mechanism. b-carotene, being highly unsaturated, seems to be consumed during autoxidation. This may suggest why the rate of lipid peroxidation is even higher in the oils with the carotenoids. **Fig 4.**

During the photoxidation with UV light at 254nm, the carotenoid extract at 6000 ppm (390 ppm b-carotene); and beta-carotene std at 400 ppm were very effective in retarding lipid oxidation. The phenolic antioxidants, the flavonoid extract at 1000 ppm and BHT at 400 ppm reduced the rate of oil oxidation to a lesser extent than the carotenoids, (**fig 3**).

The mechanism of photoxidation of the oil which initially takes place under UV light, seem to require the oxygen quenching ability of the carotenoids, in particalar b-carotene, which may indicate the occurrence of photosensitised oxidation.

In photosensitised oxidation, singlet oxygen is produced by light in the presence of sensitisers, e.g. chlorophyll, riboflavine myoglobin,

$$^1Sens \longrightarrow {}^3Sens^*$$
$$^3Sens^* + {}^3O_2 \longrightarrow {}^1O_2{}^* + {}^1Sens$$
$$^1O_2{}^* + RH \longrightarrow ROOH$$

This type of oxidation can be inhibited by b-carotene and a-tocopherol which act as quenchers either by deactivating singlet oxygen to ground state oxygen or reacting with it (Hamilton, 1994). The beta-carotene reacts with the singlet oxygen according to reaction:

$$^1O_2 + {}^1\text{b-car} \rightleftharpoons {}^3O_2 + {}^3\text{b-car}$$

It is conceivable that the early stages of light triggered deterioration involve only $^1O_2$ and molecular processes which cannot be retarded by conventional, free radical scavenging antioxidants (phenolics), (Carlsson *et al*, 1976).

The Phenolic antioxidants however still show appreciable antioxidant activity under these photosensitised reactions. This is likely so because during photoxidation, there follows the formation of the first hydroperoxides which break down to form hydroperoxy and alkoxy radicals. These being sufficiently reactive to abstract Hydrogen radicals from the linoleic acid in the oil and initiating autoxidation. Therefore the phenolic antioxidants can potentially block these initial radicals formed. This may suggests why the BHT and the flavonoids still show antioxidant activity under photoxidation of oil.

The measurement of conjugated diene in fatty acids will give an indication of the degree of unsaturation in the fatty acids in the oil. These dienes absorb strongly in the region of 230-234 nm (Tsaknis, 1996).

Oil treated with carotenoid extract at 6000 ppm had the highest protection under UV light conditions as illustrated by the lowest diene absorption. The oil treated with BHT standard and flavonoid extract under oven heating conditions showed the highest protection to oxidation as illustrated by the low diene absorbance.

Conclusions

b-carotene, capsanthin, lutein have been positively identified from the carotenoid extract of powdered paprika using TLC and UV/Visible Spectroscopy

The carotenoids showed little antioxidant properties during the autoxidation of sunflower oil at 110°C using Rancimat; b-carotene at 400 ppm has given a P.F. of 1.2 and carotenoid extract at 6000 ppm, which is equivalent to 390 ppm b-carotene gave a PF of 1.05. These Carotenoids have shown pro-oxidant activity under the dark oven autoxidation tests. However, carotenoids have shown antioxidant activity during the photoxidation of the oil with UV light at 254 nm., with peroxide values of 33.8; 20.7; 18.0; 27.5; meq. O_2 /kg oil for the control; b-carotene 400 ppm; crude carotene 6000 ppm (390ppm bet-car); and BHT 400 ppm respectively.

Flavonols and dihydroflavonols were the major flavanoid types identified in the column separated fractions from the flavonoid extract of powdered paprika using TLC and UV/Visible Spectroscopy. The flavonoids showed antioxidant activity for autoxidation oven tests, with peroxide values of 43.3; 25.2; 26.2; meq. O_2/kg oil for the control; BHT 400 ppm; and flavonoid extract at 1000 ppm respectively after 48 hours of oven heating at 55°C. These flavonoids also showed appreciable antioxidant activity under the photoxidation reactions, with peroxide values of 33.8; 27.5; 29.4; meq. O_2/kg oil for the control; BHT 400 ppm; flavonoid (crude) 1000ppm respectively under UV light at 254 nm for 48 hours.

The column separated fraction 3 had the highest concentration of dihydroflavonols and this fraction, at 2%, gave the highest protection factor of 1.81 during the Rancimat autoxidation of the sunflower oil, whilst that of BHT at 400ppm was 1.33.

Acknowledgements

I would like to express my sincere gratitude to my supervisors, Dr. John Ahmad and Dr. Mike Hole for their guidance throughout the study. I also wish to acknowledge the assistance of all technical staff of the University of Lincolnshire and Humberside for their help and patience throughout the research.

References

Buckle K A. and Rahman M. M. "Separation of Chlorophyll and Carotenoid pigments of *Capsicum* cultivars" in *J. Chromatography*, 171, (1979) 385-391.

Coultate, T. F. *"Food, The Chemistry of its components."* 3rd edition, pub. The Royal Society of Chemistry. (1996).

Carlsson D. J., Suprunchuk T. and Wiles, D. M., "Photoxidation of Unsaturated Oils, Effects of Singlet Oxygen Quenchers," in; *J.A.O.C.S., 53, 1976* (656-659)

Dondeena G. Bradley and David B. Min, "Singlet Oxygen Oxidation of Foods", in; *CRC Food* Science *and* Nutrition 31 iss. 3 *(1992).*

Fioriti and Sims in "Label as Natural (Natural Antioxidants), feature 1003, *INFORM* 1(12), 1990

Fish B. F. in "Screening and Identification of Antioxidant Producing strains in Food Borne Fungi", Holckaido University 44 (3) 141-146, 1993.

Hamilton R. J. "The Chemistry of Rancidity in Foods", in *Rancidity in Foods* pub. Chapman and Hall 1994, pp 12-14.

Hoffman G., The Chemistry of Edible fats in; "The Chemistry and Technology of Edible oils and fats and their high fat products." Academic Press, 1989 pp.3-22.

Jeorge *et al.* "Natural Antioxidants for preventing colour loss in Stored Paprika", in *Journal of Food Science*, 62(5), 1997, pp 1017.

Kanner and Frankel "Natural Antioxidants in Grapes and Wines" in *J. Agricultural Chemistry* 1991, 42, 64-69.

Ramanathan L. and Das N. P., "NaturalProducts inhibit Oxidative Rancidity in salted ground fish", in *Journal of Food Science* 58(2), 1993.

Rosalita R., De La Mar and F.J. Francis, "Carotenoid Degradation in Bleached Paprika." in; *J. of Food Science, vol 34 (1969), p* 287

St. Angelo and Allen J. "Lipid oxidation in Food", in *American Chemical Society*, May 1992.

Tsaknis John, in: *"Isolation and Identification of Antioxidants from Sideritis Euboea (mountain tea)"*, Ph.D. Thesis, April, (1996).