Food Sci. Biotechnol. Vol. 18, No. 4, pp. 904 ~ 909 (2009)

Food Science and Biotechnology

Synergistic Antioxidant Effects of Lycopene and Other Antioxidants on Methyl Linoleate Autooxidation

Youn Young Shim, Yukio Kakuda*, and John Shi[1]

Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada

[1]The Food Research Program, Agriculture and Agri-Food Canada, Guelph, Ontario N1G 5C9, Canada

Abstract The beneficial effects derived from consuming natural antioxidants may not depend on the action of an individual antioxidant, but rather on the concerted action of several antioxidants naturally present. The aim of this study was to determine the concentrations and combinations of antioxidants that can produce synergistic effects (SyEs). Quantification of the lipoperoxyl radical scavenging capacity of antioxidants was carried out in a homogeneous model system where the free radicals were produced by the oxidation of methyl linoleate, initiated by the 2,2'-azobis (2,4-dimethylvaleronitrile). The greatest SyE (2.21, $p<0.05$) was seen in mixtures of all 4 antioxidants when used with concentrations of 15 μM lycopene, 2.5 μM vitamin E, 0.16 μM vitamin C, and 10 μM β-carotene. Doubling the vitamin E concentration from 2.5 to 5.0 μM in the mixture with all 4 antioxidant reduced the SyE to 1.69 ($p<0.05$). Other combinations produced synergistic effects that ranged from 1.28 to 1.41.

Keywords: antioxidant, carotenoid, lipid peroxidation, synergistic effect, 2,2'-azobis (2,4-dimethylvaleronitrile)

Introduction

Lycopene is a bioactive carotenoid present in tomatoes, watermelon, pink grapefruits, and guava (1). Along with lycopene, many other natural antioxidants such as lutein, vitamin E (α-tocopherol), vitamin C (ascorbic acid), and β-carotene are found in ripe tomatoes and tomato products (1). The protective effect of a high intake of tomato products against the risk of a range of degenerative/chronic diseases may not depend on the action of an individual antioxidant but rather on the concerted effect of several antioxidants (2-4). The possibility that these naturally occurring antioxidants can interact with one another and enhance the total antioxidant potential has been a topic of current interest. Components in a mixed antioxidant system may inhibit oxidation reactions either additively or synergistically.

There is evidence that vitamin E prevents the degradation of β-carotene and lycopene during lipid oxidation (5). In an earlier study, Palozza and Krinsky (6) presented evidence that β-carotene acted synergistically with vitamin E during the inhibition of lipid peroxidation in multilamellar liposomes, and found that the combinations were more effective than the individual antioxidants, and that the synergistic effect was more pronounce when lycopene or lutein was present. Recently, it was reported that the antioxidant protection against porphyrin phototoxicity was synergistically enhanced in the presence of β-carotene, vitamin C, and vitamin E but not when these antioxidants were used alone (7). It was also suggested that vitamin C is able to interact with vitamin E radicals and regenerate vitamin E. Additional evidence has shown that mixtures of carotenoids were more efficient at protecting liposome against oxidation than single carotenoids, especially when lycopene and lutein were present in the mixture (8). However, in a phospholipids membrane model system, equimolar amounts of vitamin E and β-carotene showed a mild prooxidant interaction (9) while Biacs and Daood (10) studying the lipoxygenase catalyzed degradation of carotenoids reported that the combined used of vitamin C and vitamin E did not improve the antioxidative activity against co-oxidation of carotenoids. It was also reported that although water-soluble vitamin C is less reactive with lipid radicals than the hydrophobic lycopene, it was able to interact with the oxidized forms of lycopene in a time-dependent reaction to regenerate lycopene (10). This would suggest that the recycled lycopene can continue to exert its protective action against free radicals. The inhibitory effect of vitamin C on β-carotene degradation is much lower than on lycopene degradation in the co-oxidation studies. This was explained as due to the carbon-centered radical product of β-carotene inability to readily abstract a hydrogen atom from ascorbic acid. Vitamin E also inhibited the oxidation of carotenoid, but with a different mechanism than with vitamin C. Much higher levels of vitamin E are needed to protect β-carotene and the interaction of vitamin E with β-carotene is stronger than with lycopene. Although the combination of vitamin C and E exerted a mild synergistic effect compared to vitamin E alone in lowering the degradation of lycopene, the same combination caused more oxidative degradation of β-carotene. Thus the effectiveness of antioxidant action depends on the combination of antioxidants, their levels and the particular conditions under which the determinations are done.

The objective of the present investigation was to study the synergistic antioxidant properties of lycopene with other antioxidants on lipid peroxidation in an *in vitro* model system, where the fatty acid hydroperoxides were produced by lipid-soluble azo-initiator, 2,2'-azobis (2,4-dimethylvaleronitrile) (AMVN).

*Corresponding author: Tel: +1-519-824-4120 (52260); Fax: +1-519-824-6631
E-mail: ykakuda@uoguelph.ca
Received December 5, 2008; Revised February 5, 2009;
Accepted February 16, 2009

Table 1. Composition of individual antioxidants and their mixtures (μM)

Code	Experimental combinations[1)]			
	Lycopene	β-Carotene	Vitamin C[2)]	Vitamin E
S1	5			
S2	10			
S3	15			
S4		5		
S5		10		
S6			0.16	
S7				2.5
S8				5.0
S9	10	5	0.16	5.0
S10	10	10	0.16	5.0
S11	15	5	0.16	5.0
S12	15	10	0.16	5.0
S13	15	10	0.16	2.5
S14	15	10		2.5
S15	15	10	0.16	
S16	15		0.16	2.5
S17	15	10		
S18	15		0.16	
S19	15			2.5

[1)]Origin of the concentrations of each antioxidant was modified from lycopene (14), β-carotene (6,15), and vitamin E (8,14).
[2)]0.16 μM vitamin C (threshold level) was used to study effectiveness of its antioxidant action.

Materials and Methods

Materials Lycopene, vitamin E, vitamin C, β-carotene, and linoleic acid methyl ester (LAME, 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). LAME was purified on a Florisil column (1.5×30 mm, Floridin Co., Pittsburg, PA, USA) (11). AMVN was supplied by Wako Pure Chemicals Inc. (Osaka, Japan). Acetonitrile, methanol, 2-propanol, hexane, tetrahydrofuran (THF), and ethanol were purchased from Caledon Laboratories Limited (Georgetown, ON, Canada). All solvents were high-performance liquid chromatography (HPLC) grade (Fisher Scientific, Ottawa, ON, Canada).

Antioxidant preparation Table 1 shows the composition of individual antioxidants and their mixtures. The stock solution of vitamin C (5 μM) was prepared in ethanol. All other stock solutions were prepared in hexane; lycopene (31 μM), vitamin E (250 μM), and β-carotene (556 μM). All procedures were performed under dim light to protect against oxidation.

AMVN-induced lipid peroxidation Peroxyl radical scavenging activity of the antioxidants was determined by measuring the inhibition of hydroperoxide formation (12). In the present investigation, the hydrophobic azo-compound AMVN was used as the free radical initiator. AMVN can decompose to form carbon-centered radicals that react swiftly with O_2 to yield peroxyl radicals, which initiate the lipid peroxidation chain reactions. To evaluate the antioxidant activity, different levels of antioxidants, individually or in combinations, were added to 0.25 mL of LAME (308 mM stock solution) and subjected to preincubation at 20°C for 30 min in the dark using capped glass vials to avoid possible photooxidation of the unsaturated fatty acids. The volume of the preincubation solution was made up to 0.8 mL with hexane. After 30 min, 0.2 mL of AMVN (10 mM) dissolved in THF:hexane (1:2, v/v) was added to initiate the oxidation of LAME. The final volume was 1.0 mL and the final concentrations of AMVN was 2 mM, LAME was 77 mM. Immediately after adding AMVN the reaction mixture was placed inside the sample compartment of the HPLC and at regular intervals, 10 μL aliquots of each reaction mixture were injected and analyzed. The auto injector on the HPLC was set to inject each reaction mixture (7 times at 10 min intervals). The AMVN solution was prepared fresh each day and stored in a flask covered with aluminum foil, and kept in the dark at 4°C between runs. The same concentration of LAME solution without antioxidants was used as the control.

Measurement of antioxidant activity A C_{18} analytical column (Spherisorb, 5.0 μm; particle size, 4.6×250 mm; Waters, Milford, MA, USA) was used with an isocratic mobile phase consisting of acetonitrile/methanol/2-propanol (44:54:2, v/v/v) at a flow rate of 1 mL/min. At regular intervals, 10 μL aliquots of each reaction mixture were withdrawn and analyzed for methyl linoleate hydroperoxides (MeLOOH). The hydroperoxide peak was monitored at 234 nm (ε=28,000/M·cm) (5,13) every 10 min for 60 min using a photodiode array 996 detector (Waters). The *trans-trans* MeLOOH isomer eluted at 3.4 min (Fig. 1).

Calculation of %ESC, %TSC, and SyE The synergistic effects of lycopene with other antioxidants were calculated as the ratio of the percentage experimental scavenging capacity (%ESC) to the percentage theoretical scavenging capacity (%TSC). The %ESC of an antioxidant mixture is given by the following equation:

$$\%ESC=[(Abs_{control}-Abs_{sample})/Abs_{control}]\times 100$$

Where $Abs_{control}$ is the peak area of the measured hydroperoxides from LAME in the absence of antioxidant, and Abs_{sample} is the peak area of measured hydroperoxides from LAME in the presence of antioxidants. The absorbance reading at time zero was used as the blank.

The %TSC was calculated using the individual scavenging capacity results in the following equation (14):

$$\%TSC=100-[(100-ESC_{A1})\times(100-ESC_{A2}/100)\times(100-ESC_{A3}/100)\times(100-ESC_{A4}/100)]$$

Where ESC_{A1-4} are the percentage experimental scavenging capacity (%ESC) of the individual antioxidants and $(100-ESC_{A1})$ represents the fraction of the remaining hydroperoxides after 60 min of reaction. The assumption is that each antioxidant is scavenging independently and scavenging only the remaining hydroperoxide left by the other antioxidants. The synergistic effect (SyE) is calculated using the following formula (14).

$$SyE=\%ESC/\%TSC$$

Synergism is present when SyE is greater than 1.

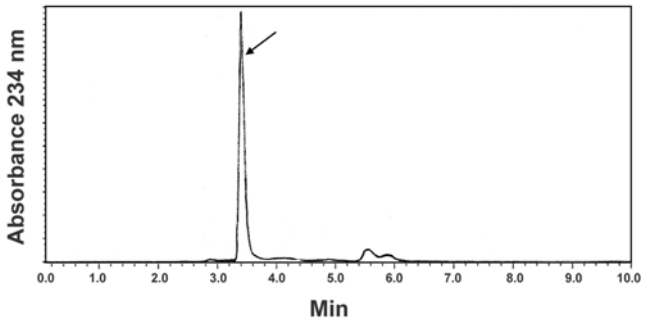

Fig. 1. Reversed-phase HPLC of methyl linoleate hydroperoxide isomers. Arrow indicates the *trans-trans* isomer.

Statistical analysis All the experiments were performed in triplicates. Student's *t*-test was used for comparison between 2 means (%ESC and %TSC), using Sigmastat software 1.0 (Jandel Corp., San Rafael, CA, USA). A difference was considered statistically significant at $p<0.05$.

Results and Discussion

Individual antioxidant capacity The composition and concentration of the individual antioxidants and their mixtures used in this study are summarized in Table 1. The final volume of each reaction mixture was 1.0 mL and the final concentration of the azo-initiator (AMVN) was 2 mM. The effects of individual antioxidants on the rate of LAME oxidation are compared in Fig. 2 and 3. The control showed the greatest rate of hydroperoxide formation increasing linearly over the 60 min incubation period. At the concentrations used in this study, all 4 antioxidants, when used alone, retarded the peroxyl radical-mediated formation of MeLOOH (Fig. 2). Vitamin E and lycopene were significantly more protective than vitamin C and β-carotene, as measured by %ESC of MeLOOH formation (Fig. 3). Because of the large %ESC produced by 5.0 μM vitamin E, this antioxidant was also examined at 2.5 μM. There was a clear induction period with the 2.5 and 5.0 μM vitamin E concentrations that lasted for 10 and 20 min, respectively. After a 20 min induction period, it appeared that the 5.0 μM vitamin E sample was depleted of 2.5 μM vitamin E and the rate of LAME oxidation began to approach the rate of the control sample (Fig. 2). After 50 and 60 min induction periods, the 15 and 10 μM lycopene concentrations were depleted 2.5 μM vitamin. The scavenging capacity of vitamin E showed a dose-dependence with the higher antioxidant concentrations resulting in a higher percentage scavenging capacity. Under the present conditions the scavenging capacities (inhibitory effects) are in the following order: 5.0 μM vitamin E>2.5 μM vitamin E> 15 μM lycopene>0.16 μM vitamin C>10 μM β-carotene (Fig. 3). The lipid-soluble antioxidant, vitamin E, was the most efficient inhibitor when the lipophilic free radical generator AMVN was used to initiate lipid peroxidation.

Antioxidant capacity of mixtures The phenomenon of cooperative interactions between lycopene, β-carotene, vitamin E, and vitamin C, was investigated by measuring their inhibitory activity against LAME oxidation individually and in combination. The theoretical and experimental scavenging capacities and their calculated synergistic effects are listed in Table 2 for 11 antioxidant combinations. Since the highest SyE values were obtained at 60 min (with only a few exceptions) these values were used for the comparison.

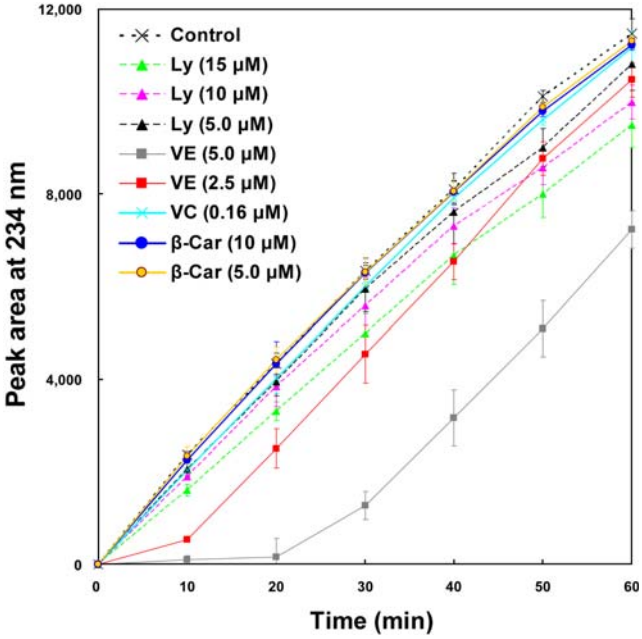

Fig. 2. AMVN-initiated lipid peroxidation. Oxidation is measured by monitoring the production of methyl linoleate hydroperoxides (MeLOOH) using reverse-phase HPLC. Where $Abs_{control}$ is the peak area of the measured hydroperoxides formed from LAME in the absence of antioxidant, and Abs_{sample} is the peak area of measured hydroperoxides formed from LAME in the presence of antioxidants. Data represent mean±SD for $n=3$. Ly= lycopene; VE=vitamin E; VC=vitamin C; β-Car=β-carotene.

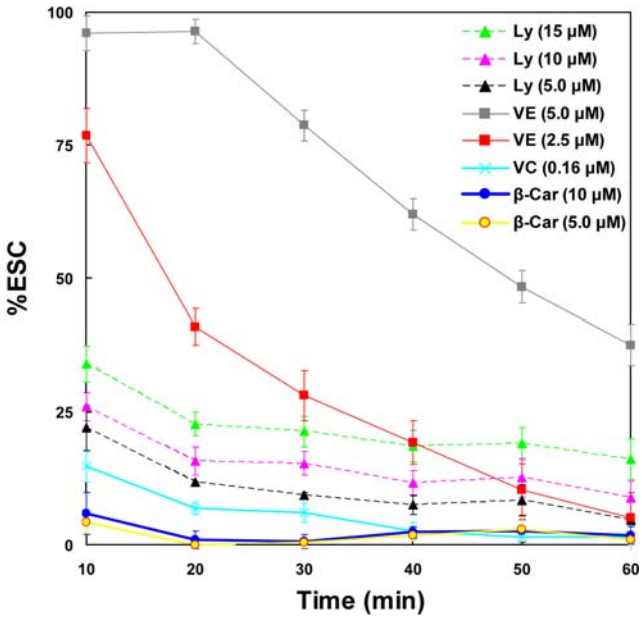

Fig. 3. Experimental scavenging capacity (%ESC) of the individual antioxidants. Data represent mean±SD for $n=3$. Ly= lycopene; VE=vitamin E; VC=vitamin C; β-Car=β-carotene.

Table 2. Synergistic effect of antioxidant mixtures at 60 min

Code	%ESC[1)]	%TSC[2)]	SyE[3)]
S9	40.1±3.7	31.7±2.5	1.25*
S10	48.9±4.0	29.6±1.9	1.39*
S11	59.1±4.9	40.2±3.9	1.47*
S12	76.3±12.1	45.2±10.2	1.69*
S13	56.4±10.0	26.1±9.9	2.21*
S14	41.8±4.9	29.6±2.9	1.41*
S15	23.9±2.1	23.3±3.9	1.03
S16	40.6±5.0	31.7±6.5	1.28*
S17	25.2±1.6	19.7±1.5	1.28**
S18	17.4±5.6	22.2±5.5	0.78
S19	33.3±2.3	28.6±4.4	1.17

[1)]%Experimental scavenging capacity; Values are the mean±SD (n=3).
[2)]%Theoretical scavenging capacity was calculated from data for single components; see Fig. 2.
[3)]Synergistic effect (SyE)=%ESC/%TSC. SyE>1: synergistic effect shown; SyE<1: no synergistic effect found. *p<0.05, **p<0.01.

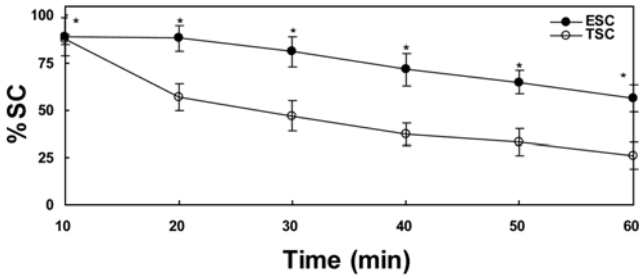

Fig. 4. Comparison of observed %experimental scavenging capacity (%ESC) and %theoretical scavenging capacity (%TSC) with the mixture of 4 antioxidants (S13) over the 60 min reaction period. Values are mean±SD. *p<0.05. S13=15 μM Ly+10 μM β-car+0.16 μM VC+2.5 μM VE.

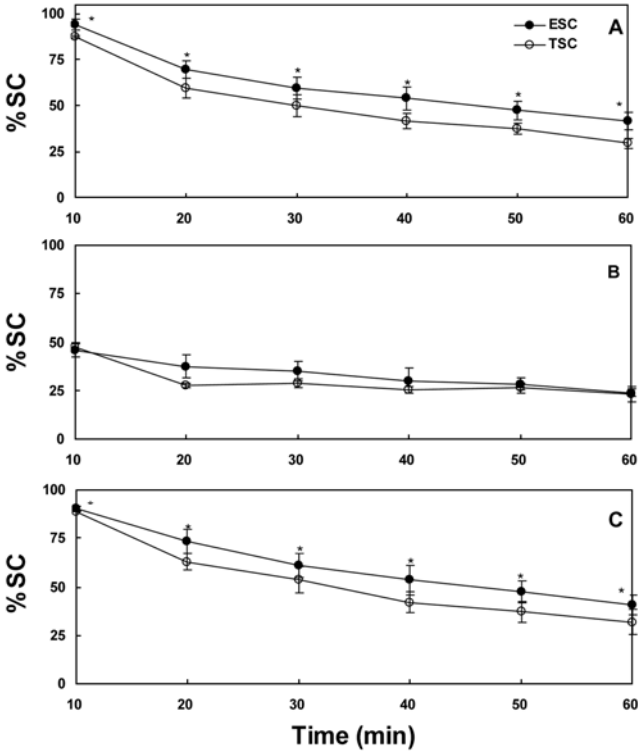

Fig. 5. Comparison of observed %experimental scavenging capacity (%ESC) and %theoretical scavenging capacity (%TSC) with the mixtures of 3 antioxidants over the 60 min reaction period. Values are mean±SD. *p<0.05. A, S14 (Ly+β-Ca+VE); B, S15 (Ly+β-Ca+VC); C, S16 (Ly+VC+VE).

Only S15, S18, and S19 did not have significant SyE values. Mixtures S12 and S13 were prepared with all 4 antioxidants and with 2 levels of vitamin E, 2.5 μM (S13) and 5.0 μM (S12). Their theoretical scavenging capacity (%TSC) values were 45.2±10.2 and 26.1±9.9% at 60 min and the experimental scavenging capacity (%ESC) values were 76.3±12.1 and 56.4±10.0%, respectively (Table 2). In previous studies, involving the inhibition of methyl linoleate oxidation by β-carotene, a direct dose dependence was observed with the antioxidant (15), however in this study, the synergistic antioxidant effects of S12 (SyE=1.69, p<0.05) was lower than S13 (SyE=2.21, p<0.05) even though S12 contained twice as much vitamin E. Similar inverse relationships have been observed by Anguelova and Warthesen (5) where higher concentrations of α-carotene and lycopene did not provide additional antioxidant activity and that β-carotene inhibited hydroperoxide formation at a concentration of 80 μg/g but did not exhibit antioxidant activity at a concentration of 160 μg/g.

Due to the large synergistic effect observed with sample S13, the same concentrations used in S13 (14 μM lycopene, 2.5 μM vitamin E, 0.16 μM vitamin C, and 10 μM β-carotene) were used in all the other mixtures where the combination of antioxidants was varied (Fig. 4). A significant but smaller synergistic effect was observed with the combination of 3 antioxidants, S14 and S16. The SyE (p<0.05) of S14 with vitamin C removed was 1.41 (Table 2 and Fig. 5A). Its %TSC was 29.6±2.9% and its %ESC was 41.8±4.9%. The results from other studies have not been in total agreement. The combination of β-carotene and vitamin E was shown to be additive when used in hexane solution (16) but when used in membrane systems there was some cooperative interaction (17). In another study, Fuhrman *et al.* (14) found that the combination of lycopene plus vitamin E, glabridin, rosmarinic acid, carnosic acid, or garlic, produced a synergistic antioxidant effect on low density lipoprotein oxidation. They showed greater synergism at the lower vitamin E concentration (5.0 μM) and no synergistic effects at 10.0 μM. Their observations suggested a superior antiatherogenic effect with a combination of antioxidants over individual effects. Mixture S16 also showed a synergistic effect (SyE=1.28, p<0.05) when lycopene, vitamin C, and vitamin E were combined, however, the effect was less than S14. When vitamin E was omitted from mixture S15, the %TSC was 23.3±3.9% and the %ESC was 23.9±2.1% (Fig. 5B) indicating that vitamin E may be playing an important role in some of these samples.

When only 2 antioxidants are present synergism was not seen, except for S17, which has a SyE value of 1.28 (Fig. 6A and Table 2). Stahl *et al.* (8) have reported a synergistic effect in the presence of lycopene and β-carotene in

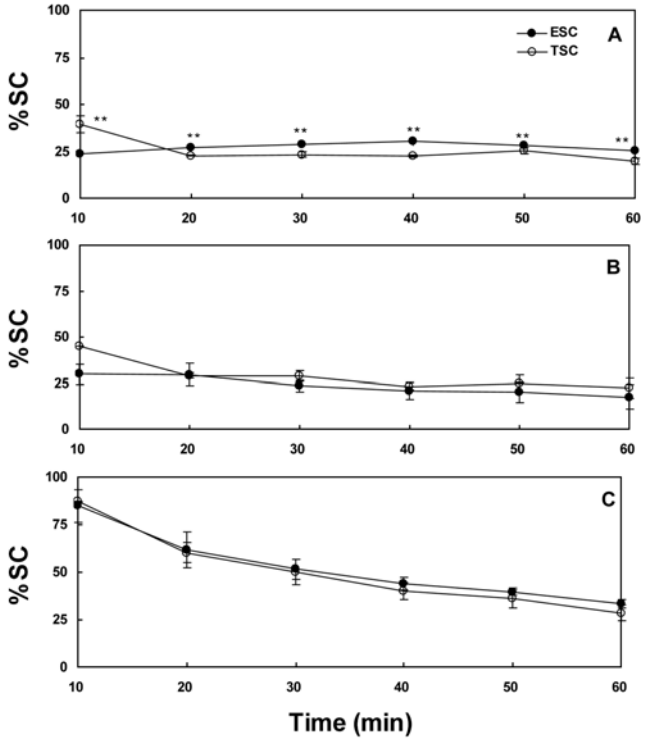

Fig. 6. Comparison of observed %experimental scavenging capacity (%ESC) and %theoretical scavenging capacity (%TSC) with the mixtures of 2 antioxidants over the 60 min reaction period. Values are mean±SD. $^{**}p<0.01$. A, S17 (Ly+β-Ca); B, S18 (Ly+VC); C, S19 (Ly+VE).

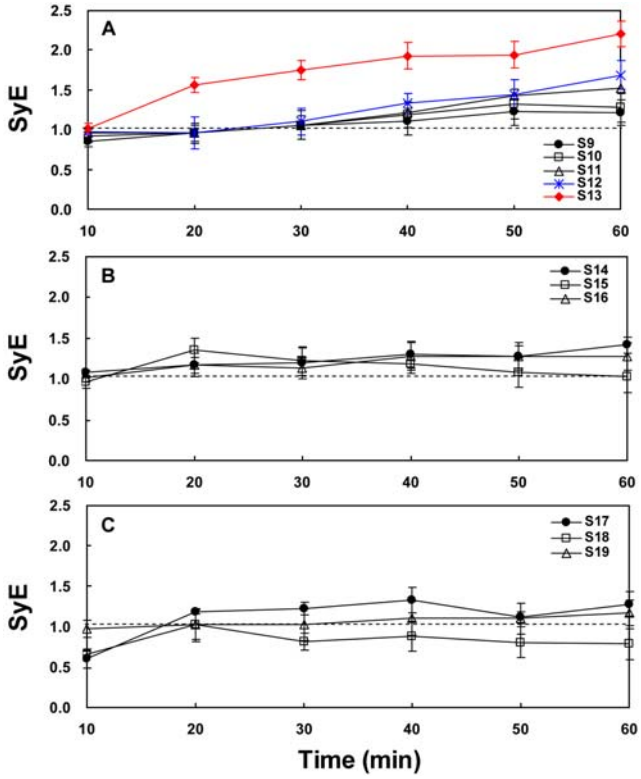

Fig. 7. Synergistic effect (SyE) of antioxidant mixture over the 60 min reaction period. Error bars represent mean±SD of at least 3 replicates. A, 4 antioxidants mixtures; B, 3 antioxidants mixtures; C, 2 antioxidants mixtures.

multilamellar liposomes. Mixture S17 exhibited a pro-oxidant effect after 20 min incubation; this was not seen in any of the other antioxidant mixtures. The reason for this has not been determined but the low vitamin C levels (0.16 μM) used this study may have resulted in its rapid depletion allowing the formation of carotenoid peroxyl radical, which can attack unsaturated lipids and continue the oxidation reaction (15). In those mixtures with carotenoids and vitamin C with no vitamin E (S15 and S17), a peak in the SyE value can be seen at 20 min (Fig. 7B and 7C). This may be in support of the supposition that vitamin C is regenerating the carotenoids during the early stages producing a peak in the SyE plot at 20 min but is depleted due to its low concentration and allowing the formation of the carotenoid radicals.

The largest synergistic effects seen in this study was produced by mixtures containing 4 antioxidants (S12 and S13). Mixture S13 with the lower vitamin E concentration was 2.2 times more effective than the additive effects of the individual antioxidants. The synergistic effects seen when all 4 antioxidants were present (S13) increased steadily over time. With 4 antioxidants, there are many possible cooperative interactions that can spare, regenerate, or partition in separate phases to enhance the antioxidant activities. Several studies have shown vitamin C to be a powerful water-soluble antioxidant that can regenerate carotenoids radical cations, suggesting a direct interaction of carotenoid radical cations with vitamin C, even though the parent carotenoid is located in a hydrophobic environment (18,19). Niki *et al.* (17) reported that vitamin C can regenerate vitamin E in lipid systems and vitamin E, β-carotene may exert a cooperative effect but β-carotene and vitamin C do not appear to interact synergistically. The synergistic effects between the carotenoid zeaxanthin and vitamin E have been reported by Wrona *et al.* (20) in the photosensitized lipid peroxidation of liposomes as well as the regeneration of vitamin E by green tea polyphenols (21). Yeum *et al.* (22) have also observed that water-soluble and the fat-soluble antioxidants present in separate phases are able to interact with each other. Therefore, when evaluating the antioxidant properties of antioxidants, the composition of the antioxidants, the local environment, the oxidizing substrate and the potential interactions between individual antioxidants should be considered.

In conclusion, under the conditions used in this study vitamin E showed the greatest antioxidant activity in retarding the formation of MeLOOH, lycopene had the second highest scavenging capacity followed by vitamin C and β-carotene when used individually. Synergistic antioxidant effects were observed when lycopene was used in combination with vitamin C, vitamin E, and β-carotene. The greatest synergistic effect was observed when all 4 antioxidants were used in the following concentrations: 15 μM lycopene, 2.5 μM vitamin E, 0.16 μM vitamin C, and 10 μM β-carotene. The synergistic effect is lost when vitamin E is removed but not when vitamin C or β-carotene was removed from the mixture of 4 antioxidants. Lycopene when combined with low levels of vitamin C

produced a prooxidant effect, no effect when combined with vitamin E and a synergistic effect when mixed with β-carotene.

It appears that the synergistic effect is dependent on the combination of antioxidants used as well as their concentrations. These results provide additional evidence that mixtures of antioxidants can have a greater effect than the sum of the individual antioxidants.

Acknowledgment

The authors wish to thank the Food Research Program, Agriculture and Agri-Food Canada (AAFC), Guelph.

References

1. Shi J, Kakuda Y, Yeung D. Antioxidative properties of lycopene and other carotenoids from tomatoes: Synergistic effects. BioFactors 21: 203-210 (2004)
2. Klipstein-Grobusch K, Launer LJ, Geleijnse JM, Boeing H, Hofman A, Witteman JCM. Serum carotenoids and atherosclerosis: The Rotterdam study. Atherosclerosis 148: 49-56 (2000)
3. Rao AV, Agarwal S. Role of antioxidant lycopene in cancer and heart disease. J. Am. Coll. Nutr. 19: 563-569 (2000)
4. Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. Intake of carotenoids and retinol in relation to risk of prostate cancer. J. Natl. Cancer I. 87: 1767-1776 (1995)
5. Anguelova T, Warthesen J. Degradation of lycopene, α-carotene, and β-carotene during lipid peroxidation. J. Food Sci. 65: 71-75 (2000)
6. Palozza P, Krinsky NI. β-Carotene and vitamin E are synergistic antioxidants. Arch. Biochem. Biophys. 297: 184-187 (1992)
7. Böhm F, Edge R, Foley S, Lange L, Truscott TG. Antioxidant inhibition of porphyrin-induced cellular phototoxicity. J. Photoch. Photobio. B. 65: 177-183 (2001)
8. Stahl W, Junghans A, de Boer B, Drionina ES, Briviba K, Sies H. Carotenoid mixtures protect multilamellar liposomes against oxidative damage: Synergistic effects of lycopene and lutein. FEBS Lett. 427: 305-308 (1998)
9. Stratton SP, Liebler DC. Determination of singlet oxygenspecific versus radical-mediated lipid peroxidation in photosensitized oxidation of lipid bilayers: Effect of beta-carotene and alpha-tocopherol. Biochemistry 36: 12911-12920 (1997)
10. Biacs PA, Daood HG. Lipoxygenase-catalysed degradation of carotenoids from tomato in the presence of antioxidant vitamins. Biochem. Soc. T. 28: 839-845 (2000)
11. Terao J, Nagao A, Park D, Lim PB. Lipid hydroperoxide assay for antioxidant activity of carotenoids. Method Enzymol. 213: 454-460 (1992)
12. Tesoriere L, Ciaccio M, Bongiorno A, Riccio A, Pintaudi AM, Liverea MA. Antioxidant activity of all-trans-retinol in homogeneous solution and in phosphatidylcholine liposomes. Arch. Biochem. Biophys. 307: 217-223 (1993)
13. Chan H, Levett G. Autoxidation of methyl linoleate. Separation and analysis of isomeric mixtures of methyl linoleate hydroperoxides and methyl hydroxylinoleates. Lipids 12: 99-104 (1977)
14. Fuhrman B, Volkova N, Rosenblat M, Aviram M. Lycopene synergistically inhibits LDL oxidation in combination with vitamin E, glabridin, rosmarinic acid, carnosic acid, or garlic. Antioxid. Redox Sign. 2: 491-506 (2000)
15. Tsuchihashi H, Kigoshi M, Iwatsuki M, Niki E. Action of β-carotene as an antioxidant against lipid peroxidation. Arch. Biochem. Biophys. 323: 137-147 (1995)
16. Palozza P, Krinsky NI. The inhibition of radical-initiated peroxidation of microsomal lipids by both α-tocopherol and β-carotene. Free Radical Bio. Med. 11: 407-414 (1991)
17. Niki E, Noguchi N, Tsuchihashi H, Gotoh N. Interaction among vitamin C, vitamin E, and β-carotene. Am. J. Clin. Nutr. 62: 1322S-1326S (1995)
18. Burke M, Edge R, Land EJ, Truscott TG. Characterisation of carotenoid radilcal cations in liposomal environments: Interaction with vitamin C. J. Photoch. Photobio. B. 60: 1-6 (2001)
19. Mortensen A, Skibsted LH, Truscott TG. The interaction of dietary carotenoids with radical species. Arch. Biochem. Biophys. 385: 13-19 (2001)
20. Wrona M, Korytowski W, Rozanowska M, Sarna T, Truscott TG. Cooperation of antioxidants in protection against photosensitized oxidation. Free Radical Bio. Med. 35: 1319-1329 (2003)
21. Aldini G, Yeum KJ, Carini M, Krinsky NI, Russell RM. (-)-Epigallocatechin-(3)-gallate prevents oxidative damage in both the aqueous and lipid compartments of human plasma. Biochem. Bioph. Res. Co. 302: 409-414 (2003)
22. Yeum KJ, Aldini G, Chung HY, Krinsky NI, Russell RM. The activities of antioxidant nutrients in human plasma depend on the localization of attacking radical species. J. Nutr. 133: 2688-2691 (2003)