

## The Synergistic Effect of Daidzein and $\alpha$ -Tocopherol or Ascorbic Acid on Microsome and LDL Oxidation

HEYA WANG<sup>1,2</sup>, MASIBO WANYONYI MARTIN<sup>2</sup> and SHIAN YIN<sup>1</sup>

<sup>1</sup>National Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing, P. R. China; <sup>2</sup>School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, P. R. China

### Abstract

WANG H., MARTIN M.W., YIN S. (2010): **The synergistic effect of daidzein and  $\alpha$ -tocopherol or ascorbic acid on microsome and LDL oxidation.** Czech J. Food Sci., **28**: 385–391.

Isoflavone daidzein brings potential health benefits. Its antioxidant properties are considered to be responsible in part for its protective effects. We investigated the antioxidant effects of daidzein and its interactive effects with  $\alpha$ -tocopherol or ascorbic acid on Fe<sup>2+</sup>/ascorbate-induced oxidation of rat liver microsomes and copper-induced human low-density lipoprotein (LDL) oxidation. Although the inhibitory effect of daidzein on lipid peroxidation in microsome was weak, it effectively prevented LDL against oxidative modification by prolonging the lag time, decreasing the propagating rate, and suppressing malonaldehyde (MDA) and carbonyls formation. When daidzein was combined with  $\alpha$ -tocopherol in microsomes oxidation and with ascorbic acid in LDL oxidation, the protection was significantly greater than the calculated additive effect of the two individual actions. Thus, daidzein can protect LDL from oxidative modification, and its combination with nutrients may be superior to the action of it alone. These results can help to get a better understanding of the interactions of different antioxidants *in vivo*.

**Keywords:** antioxidant effects; synergism; daidzein; ascorbic acid;  $\alpha$ -tocopherol; LDL; microsome

Isoflavones, which are found almost exclusively in leguminous plants such as soy or *Pueraria lobata*, are a group of flavonoids which were classified as phytoestrogens with structural similarities to estrogen (MANACH *et al.* 2004). Genistein and daidzein are the two most important isoflavones contained in about equal quantities in soy (MANACH *et al.* 2005). Accumulating evidence from *in vivo* studies suggests that isoflavones may have an impact on cardiovascular diseases, cancers, and osteoporosis (OMONI & ALUKO 2005; COOKE 2006; CHAN *et al.* 2007; KURAHASHI *et al.* 2007; IWASAKI *et al.* 2008), and that their antioxidant activity might be responsible for their protective effects (PIETTA 2000). It has been reported that isoflavones have radical scavenging capacity and

antioxidative effects *in vitro* and *in vivo* and can protect tissues against oxidative damage (MAHN *et al.* 2005; BORRAS *et al.* 2006; RUFER & KULLING 2006).

Regular consumption of fruits and vegetables may provide desirable health benefits and play important roles in the prevention of some chronic diseases, such as aging, cancer, neurodegenerative diseases, and atherosclerosis, while purified phytonutrients from these fruits and vegetables do not bring the same benefits (VATTEM *et al.* 2005). Therefore, it was suggested that the additive and synergistic effects of different antioxidants are responsible for their potent antioxidant and diseases preventive effects (LIU 2004). Isoflavones may exert protection effects in combination with dietary antioxidant nutrients,

especially with  $\alpha$ -tocopherol and ascorbic acid, two widely distributed antioxidant nutrients from food. The information on antioxidant interactions between plant isoflavones and nutrients would provide a better understanding of the protecting effects of complex food.

In this study, we describe the antioxidant activity of daidzein and its interplay with  $\alpha$ -tocopherol or ascorbic acid using  $\text{Fe}^{2+}$ /ascorbate-induced oxidation of rat liver microsomes and copper-induced LDL oxidation as model systems. The possible mechanisms that may account for the observations obtained are discussed.

## MATERIALS AND METHODS

**Materials.** Daidzein (Da > 98%) extracted from *Pueraria lobata* was purchased from Ankang Heye Bioengineering Co., Ltd. (Shanxi, China).  $\alpha$ -Tocopherol (TO > 99.8%), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferrozine, and malonaldehyde (MDA) were obtained from Sigma (St. Louis, USA). Thiobarbituric acid (TBA) was obtained from Merck (Darmstadt, Germany). Ascorbic acid (AA > 99.9%), butylated hydroxytoluene (BHT), trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), and 4-dinitrophenylhydrazine (DNPH) were obtained from Biosino Biotechnology Co., Ltd. (Beijing, China).

**Reducing capacity.** The reductive potential of antioxidants was determined according to the method of YEN *et al.* (1995). The antioxidants diluted in 1.5 ml phosphate buffer (0.2M, pH 6.6) were mixed with 0.5 ml potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] (final concentration of 2.5 mg/ml), followed by incubation at 50°C for 20 minutes. The reaction was terminated by adding 0.5 ml of 200 g/l TCA solution and the mixture was centrifuged for 10 min at  $1000 \times g$  (CS-15R, Beckman, Brea, USA). The supernatant was mixed with 0.03 ml  $\text{FeCl}_3$  solution (1 mg/ml), and the absorbance was measured at 700 nm (UV-1600 spectrophotometer, RUILI, Beijing, China).

**Metal chelating activity.** The chelating of ferrous ions of the antioxidants was estimated by the method of DECKER and WELCH (1990) with a slight modification. Briefly, the antioxidants were diluted with 1 ml ethanol and mixed with 40  $\mu\text{l}$  of  $\text{FeCl}_2$  (40  $\mu\text{M}$ ) and 40  $\mu\text{l}$  ferrozine (200  $\mu\text{M}$ ), followed by standing at room temperature for 15 minutes. The absorbance of the solution was measured at 562 nm.

**DPPH radical-scavenging activity.** The DPPH radical-scavenging activity of the antioxidants was

determined by the method described by BLOIS (1958) with a slight modification. Daidzein and TO were diluted with ethanol and added to ethanol solution of DPPH (40  $\mu\text{M}$ ). The mixtures were left at room temperature in the dark for 60 min and the absorbance at 515 nm was determined.

**Inhibition of lipid peroxidation in rat liver microsomes.** Liver microsomes of rats were isolated as described previously (CAI *et al.* 2003) and protein content was determined by the method of Lowry (LOWRY *et al.* 1951). Microsomes were suspended in 0.1M Tris-HCl buffer (pH 7.5) to the final concentration of 0.5 mg protein/ml. Daidzein and TO were dissolved in ethanol and diluted with 0.1M Tris-HCl buffer. The final concentration of ethanol in the suspension was lower than 1% (v/v). Microsomes were pre-incubated at 37°C for 15 min before the initiation of oxidation with antioxidants or an equivalent volume of solvent as the control. Oxidation was initiated by the addition of  $\text{Fe}^{2+}$  and ascorbic acid of final concentrations of 5  $\mu\text{M}$  and 50  $\mu\text{M}$ , respectively. Microsomal suspension was incubated at 37°C for 30 minutes. The formation of thiobarbituric acid reactive substances (TBARS) was used to evaluate lipid peroxidation in microsomes (BUEGE & AUST 1978). TBARS in the supernatant was determined at 532 nm with MDA as the standard.

**Inhibition of copper-induced oxidative modification in LDL.** Human blood from healthy donors was obtained from a hospital and LDL was separated by sequential ultracentrifugation (HAVEL *et al.* 1955). LDL protein was determined using Lowry's method (LOWRY *et al.* 1951). The effects of antioxidants on the lag time of copper-induced LDL oxidation were assessed according to the method described previously (ESTERBAUER *et al.* 1989) with a slight modification. LDL (50  $\mu\text{g}$  protein/ml) was oxidised in 10mM PBS (pH 7.4), the reaction being initiated with 5  $\mu\text{M}$   $\text{CuSO}_4$ . The formation of conjugated dienes was continually monitored by measuring the absorbance at 234 nm and 37°C. The results of LDL oxidation were expressed as the lag time and propagation rate, defined as the intercept of the slopes for the lag and propagation phases and the slope of the propagation phase in the diene-time plot, respectively (ESTERBAUER *et al.* 1989). Daidzein was dissolved in methanol and AA was dissolved in distilled water, and they were subsequently diluted with PBS to obtain the selected concentrations. The final concentration of methanol was 0.5% and the solvent control showed

that it had no apparent influence on LDL oxidation. The antioxidants were incubated with LDL at 37°C for 15 min before the initiation of oxidation.

LDL oxidation was also monitored by removing aliquots of the reaction mixture at indicated times and measuring the formation of MDA and carbonyl groups. Oxidation was terminated by the addition of 20 µM BHT and 20 µM EDTA and the reaction mixture was then cooled to 4°C. MDA formation was monitored by measuring the content of TBARS (BUEGE & AUST 1978). Carbonyl groups in oxidised LDL were determined according to HAZELL *et al.* (1994). An average molar absorption coefficient of 22 000 M<sup>-1</sup>cm<sup>-1</sup> was used to estimate the carbonyl content.

**Statistics.** All results were reported as means ± standard deviation ( $n = 3$ ). EC<sub>50</sub> values calculated denoted the concentration of the sample required to chelate 50% ferrous ions, to scavenge 50% of DPPH radicals, or to inhibit 50% lipid peroxidation in rat liver microsomes. The significance of the differences between the treatments and control was tested by *t*-tests. 2 × 2 factorial designs were performed to determine the significance of the interaction between daidzein and ascorbic acid or α-tocopherol, and the data were analysed using ANOVA (SPSS statistical software).  $P < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

### Reducing capacity, metal chelating activity and DPPH radical-scavenging activity

The reducing capacity ( $\Delta$  absorbance at 700 nm) of daidzein was negligible in concentrations of up to 60 µM, while TO and AA revealed a significant reducing capacity at the concentration of 20 µM (Figure 1). In the same way, daidzein showed a much

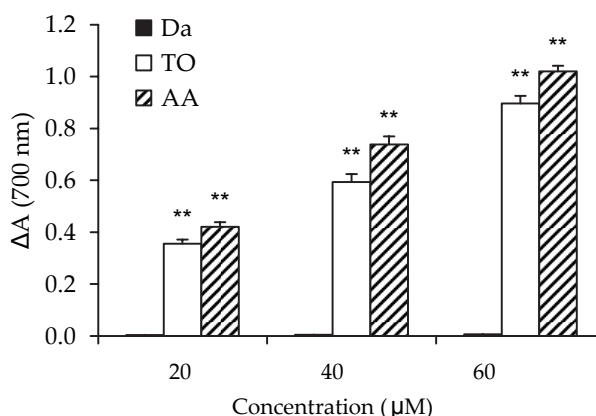


Figure 1. Reducing capacity of daidzein, α-tocopherol and ascorbic acid (\*\* $P < 0.01$  compared with the control, *t*-test)

lower DPPH radical-scavenging activity than TO and AA (Table 1), which indicates that daidzein has a low electron-donating ability (BLOIS 1958). Daidzein, TO, and AA had no metal chelating activity at concentrations of up to 100 µM, while metal chelator EDTA chelated Fe<sup>2+</sup> at the ratio of about 1:1 (Table 1).

Our results show that daidzein has a low reducing capacity, DPPH-radical scavenging activity and metal chelation ability, indicating its low reactivity with radicals and oxidants. These findings are in agreement with other studies demonstrating that daidzein has relative high redox potential, low radical-scavenging activity, and negligible chelating ability (KUO *et al.* 1998; GUO *et al.* 2002; CHEN *et al.* 2005b; FIRUZI *et al.* 2005).

### Inhibition of lipid peroxidation in microsome oxidation

In Fe<sup>2+</sup>/ascorbate initiated microsomes oxidation, the EC<sub>50</sub> value of daidzein (424 ± 1 µM) is much higher than that of TO (72.3 ± 2.1 µM) (Table 1),

Table 1. EC<sub>50</sub> values of daidzein, α-tocopherol and ascorbic acid for metal chelating activity, DPPH radical scavenging activity and inhibitory activity in microsome oxidation

Compounds	Metal chelating (µM) <sup>a</sup>	DPPH (µM) <sup>a</sup>	Microsome oxidation (µM) <sup>b</sup>
Daidzein	> 100	956 ± 6	424 ± 1
α-Tocopherol	> 100	10.3 ± 0.2	72.3 ± 2.1
Ascorbic acid	> 100	11.0 ± 0.5	N.D.
EDTA	21.0 ± 0.6	N.D.	N.D.

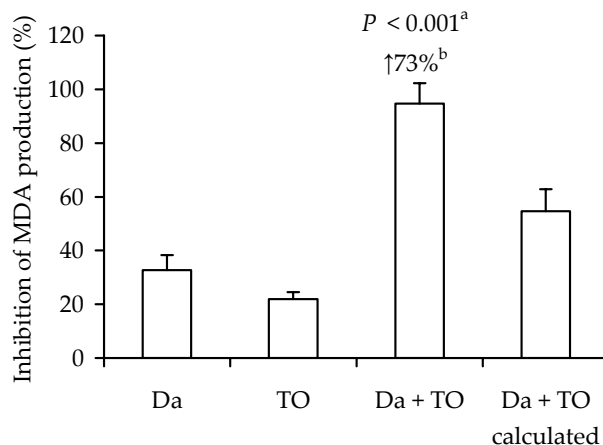
<sup>a</sup>EC<sub>50</sub> values were concentrations required for decreasing the initial ferrous ions or DPPH concentration by 50%; <sup>b</sup>EC<sub>50</sub> values were concentrations of antioxidants that inhibited microsome oxidation by 50%. N.D. – not determined

which indicates that daidzein is a weak antioxidant in liver microsomes oxidation and its activity is much lower than that of  $\alpha$ -tocopherol. However, the difference between daidzein and  $\alpha$ -tocopherol in the inhibition of microsomes oxidation was smaller than that in reducing power or DPPH-radical scavenging activity (Figure 1, Table 1). This indicates that daidzein must exert antioxidant activity by some mechanisms other than free radical-scavenging and metal chelating mechanisms.

Figure 2 compares the observed inhibition effect resulting from daidzein and TO co-incubation with the expected (calculated) sum of the values observed for the separate daidzein and TO treatments. A significant interaction ( $P < 0.001$ ) occurred between daidzein and TO. The percentage of inhibition of MDA production was by 73% greater than the expected additive value with the combination of  $300\mu\text{M}$  daidzein and  $45\mu\text{M}$  TO (Figure 2), which suggests that daidzein synergistically interacted with  $\alpha$ -tocopherol in preventing lipid peroxidation of microsomes.

### Inhibition of LDL oxidation modification

Daidzein prolonged the lag phase and decreased the propagation rate of copper-induced human LDL oxidation in a dose-dependent manner (Figure 3). Daidzein at concentrations of  $1.25\mu\text{M}$ ,  $2.5\mu\text{M}$ ,  $5\mu\text{M}$  significantly prolonged the lag time of LDL oxidation, respectively, by 17%, 48%, and 82% and reduced the propagation rate by 16%, 260%, and 35%, respectively, compared with the control. The effect of AA on lag time was greater than



<sup>a</sup> $P$  indicates the synergistic effects and  $P < 0.05$  means significant synergy, ANOVA; <sup>b</sup>the observed synergy greater than the calculated sums of the daidzein and  $\alpha$ -tocopherol treatment alone

Figure 2. The synergistic effect of daidzein and  $\alpha$ -tocopherol on the inhibition of  $\text{Fe}^{2+}$ /ascorbate induced microsome oxidation

that of daidzein, with the lag time increasing by 33%, 100%, and 179% respectively, but it had no effect on the propagation rate of LDL oxidation (Figure 3). These results indicate that daidzein inhibition effects on LDL oxidation are much greater than those on microsomes oxidation. The copper-induced LDL oxidation was also evaluated by monitoring the formation of MDA and protein carbonyls (Figure 4). MDA and carbonyls formation was strongly delayed down by  $2.5\mu\text{M}$  daidzein or  $2.5\mu\text{M}$  AA, compared with the control LDL. Thereby, daidzein effectively increased the resistance of human LDL to lipid and protein oxidation

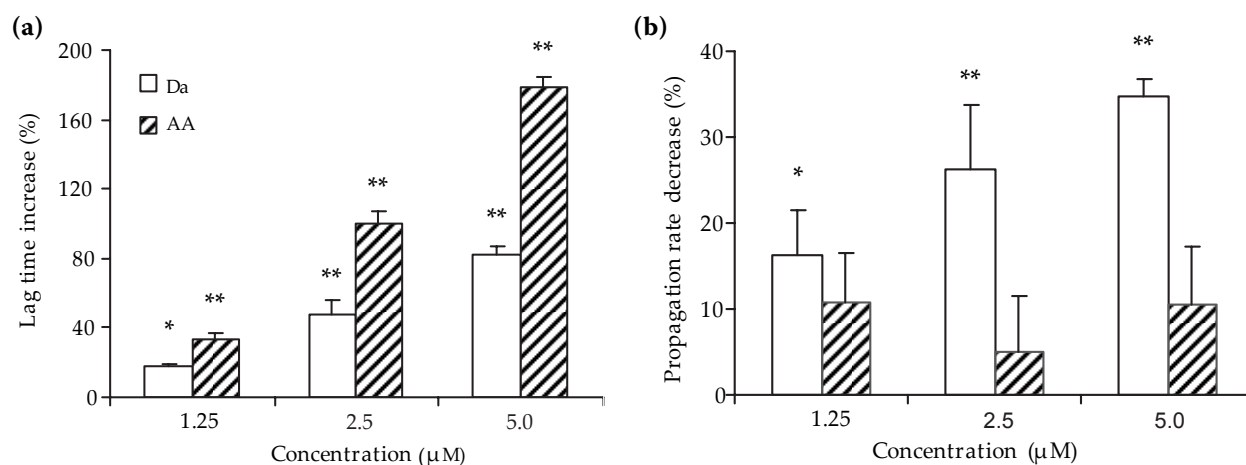


Figure 3. Effects of daidzein and ascorbic acid on lag time increase (a) and propagation rate decrease (b) of copper-induced LDL oxidation (\* $P < 0.05$  vs control; \*\* $P < 0.01$  compared with the control,  $t$ -test)

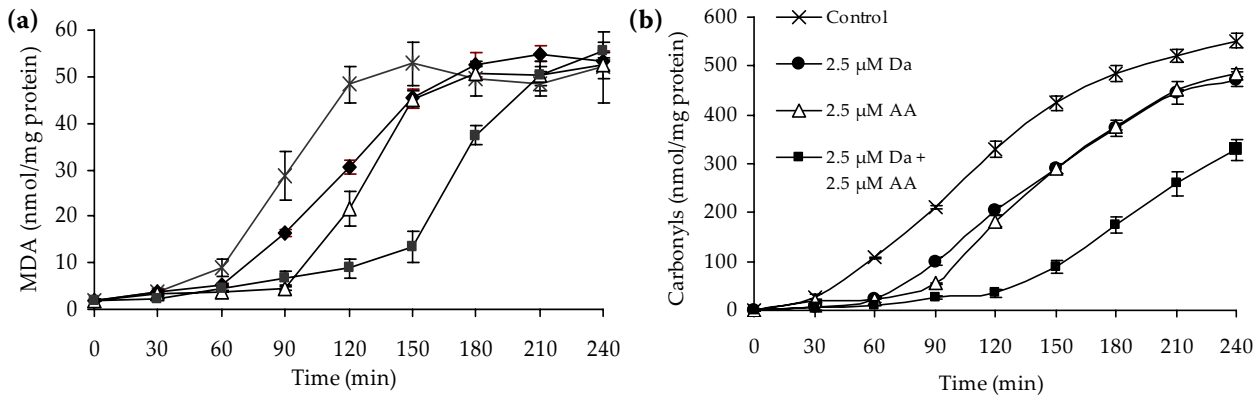


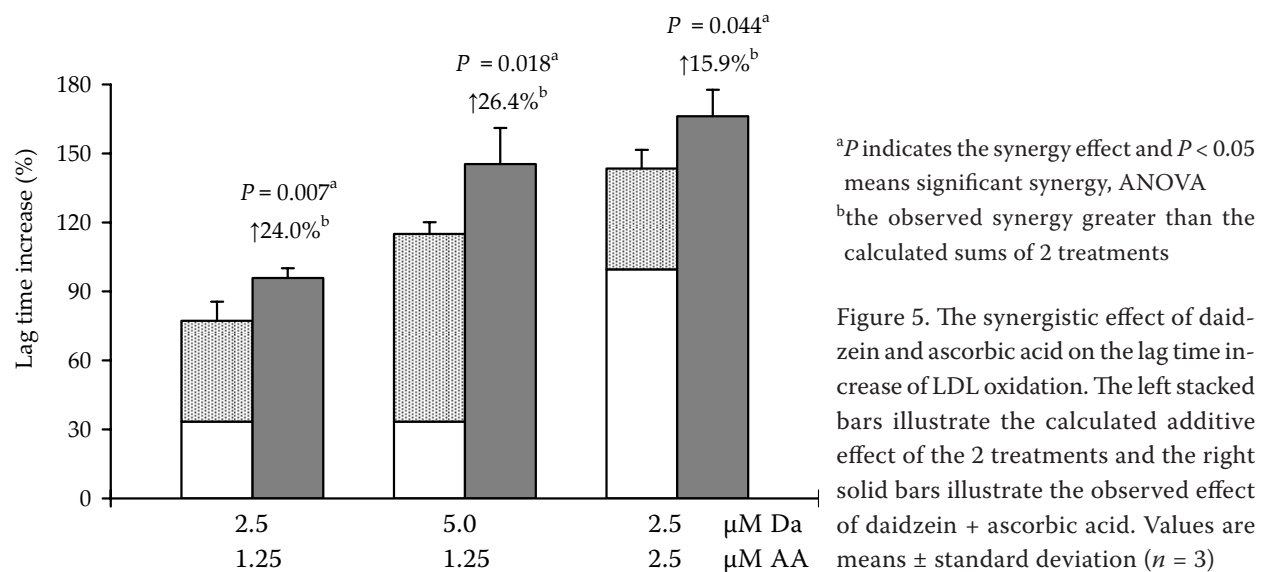
Figure 4. Kinetics of copper-induced LDL oxidation as measured by the formation of MDA (a) and carbonyls (b) in control LDL and LDL in the presence of daidzein and/or ascorbic acid

in a dose-dependent fashion, which is in agreement with several reports about the antioxidant activity of isoflavones (VAYA *et al.* 2003; RUFER & KULLING 2006; JEFREMOV *et al.* 2007).

VAYA *et al.* (2003) reported that daidzein and genistein strongly inhibited LDL oxidation with  $\text{Cu}^{2+}$  as inducer, but showed only a weak effect with 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH) as inducer. In the copper-induced oxidation of LDL,  $\text{Cu}^{2+}$  specifically binds to apoprotein B (apo-B) and is reduced to  $\text{Cu}^+$ , and a marked conformational modification of apo-B takes place during the lag phase (SEVANIAN & URSINI 2000). Given that daidzein has a low reactivity with metal ions (Figure 1, Table 1), the antioxidant action of daidzein could be explained partially by its binding with the apo-B domain in LDL particle structure, preventing the interaction of copper and LDL. This explaina-

tion is in agreement with other studies (FILIFE *et al.* 2001; CHEN *et al.* 2007).

Significant interactions in the lag time increase were observed when 2.5 μM daidzein was combined with 1.25 μM or 2.5 μM of AA ( $P < 0.05$ ), and when 5 μM daidzein was combined with 1.25 μM of AA ( $P < 0.05$ ). The effects of the three combinations were greater than the additive effects of two individual treatments, respectively (Figure 5). And when daidzein and AA were simultaneously added, the inhibition of MDA and carbonyls formation was also greater than the calculated additive inhibitory effect of the separate daidzein and AA treatments. Similar effects of flavonoids and TO or AA in preventing LDL oxidation (CHEN *et al.* 2005a, 2007; YEOMANS *et al.* 2005), and of flavonoids and urate in preventing plasma oxidation (FILIFE *et al.* 2001) were observed by other researchers. The underlying



<sup>a</sup> $P$  indicates the synergy effect and  $P < 0.05$  means significant synergy, ANOVA

<sup>b</sup>the observed synergy greater than the calculated sums of 2 treatments

Figure 5. The synergistic effect of daidzein and ascorbic acid on the lag time increase of LDL oxidation. The left stacked bars illustrate the calculated additive effect of the 2 treatments and the right solid bars illustrate the observed effect of daidzein + ascorbic acid. Values are means  $\pm$  standard deviation ( $n = 3$ )

ing mechanisms of the interaction have not been well established, although the regeneration of one antioxidant by another was proposed as contributing to this effect (FILIPE *et al.* 2001; YEOMANS *et al.* 2005). On the other hand, it is possible that the combination of different antioxidants following different mechanisms has a higher antioxidant effect than shown by the sum of the individual effects (YEOMANS *et al.* 2005; MILDE *et al.* 2007). These pieces of evidence suggest that a complex food containing isoflavone and antioxidant nutrients may exert higher health benefits than the purified isoflavone supplements.

## CONCLUSIONS

The results of the present study indicate that, although the inhibitory effect of daidzein on lipid peroxidation in microsomes was weak, daidzein effectively prevented copper-induced LDL oxidation by prolonging the lag time, decreasing the propagating rate, and suppressing malonaldehyde (MDA) and carbonyls formation. When daidzein was combined with  $\alpha$ -tocopherol in microsomes oxidation, and when it was combined with ascorbic acid in LDL oxidation, the protection was significantly greater than the calculated additive effect of two individual actions. Our results on the *in vitro* effects of the antioxidant combinations, although not directly applicable to humans, can help to get a better understanding of the *in vivo* synergy of different antioxidants from food.

## References

- BLOIS M.S. (1958): Antioxidant determinations by the use of a stable free radical. *Nature*, **181**: 1199–1200.
- BORRÁS C., GAMBINI J., GÓMEZ-CABRERA M.C., SASTRE J., PALLARDÓ F.V., MANN G.E., VIÑA J. (2006): Genistein, a soy isoflavone, up-regulates expression of antioxidant genes: Involvement of estrogen receptors, ERK1/2, and NF $\kappa$ B. *Faseb Journal*, **20**: 2136–2138.
- BUEGE J.A., AUST S.D. (1978): Microsomal lipid peroxidation. *Methods in Enzymology*, **52**: 302–310.
- CAI Y.J., FANG J.G., MA L.P., YANG L., LIU Z.L. (2003): Inhibition of free radical-induced peroxidation of rat liver microsomes by resveratrol and its analogues. *Biochimica et Biophysica Acta*, **1637**: 31–38.
- CHAN Y.H., LAU K.K., YIU K.H., LI S.W., CHAN H.T., TAM S., SHU X.O., LAU C.P., TSE H.F. (2007): Isoflavone intake in persons at high risk of cardiovascular events: implications for vascular endothelial function and the carotid atherosclerotic burden. *American Journal of Clinical Nutrition*, **86**: 938–945.
- CHEN C.Y., MILBURY P.E., LAPSLEY K., BLUMBERG J.B. (2005a): Flavonoids from almond skins are bioavailable and act synergistically with vitamins C and E to enhance hamster and human LDL resistance to oxidation. *Journal of Nutrition*, **135**: 1366–1373.
- CHEN Y.C., SUGIYAMA Y., ABE N., KURUTO-NIWA R., NOZAWA R., HIROTA A. (2005b): DPPH radical-scavenging compounds from dou-chi, a soybean fermented food. *Bioscience, Biotechnology, and Biochemistry*, **69**: 999–1006.
- CHEN C.Y., MILBURY P.E., CHUNG S.K., BLUMBERG J. (2007): Effect of almond skin polyphenolics and quercetin on human LDL and apolipoprotein B-100 oxidation and conformation. *Journal of Nutritional Biochemistry*, **18**: 785–794.
- COOKE G.M. (2006): A review of the animal models used to investigate the health benefits of soy isoflavones. *Journal of AOAC International*, **89**: 1215–1227.
- DECKER E.A., WELCH B. (1990): Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and Food Chemistry*, **38**: 674–677.
- ESTERBAUER H., STRIEGL G., PUHL H., ROTHENEDER M. (1989): Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. *Free Radical Research Communications*, **6**: 67–75.
- FILIPE P., LANCA V., SILVA J.N., MORLIERE P., SANTUS R., FERNANDES A. (2001): Flavonoids and urate antioxidant interplay in plasma oxidative stress. *Molecular and Cellular Biochemistry*, **221**: 79–87.
- FIRUZI O., LACANNA A., PETRUCCI R., MARROSU G., SASO L. (2005): Evaluation of the antioxidant activity of flavonoids by “ferric reducing antioxidant power” assay and cyclic voltammetry. *Biochimica et Biophysica Acta*, **1721**: 174–184.
- GUO Q., RIMBACH G., MOINI H., WEBER S., PACKER L. (2002): ESR and cell culture studies on free radical-scavenging and antioxidant activities of isoflavonoids. *Toxicology*, **179**: 171–180.
- HAVEL R.J., EDER H.A., BRAGDON J.H. (1955): The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *Journal of Clinical Investigation*, **34**: 1345–1353.
- HAZELL L.J., VAN DEN BERG J.J., STOCKER R. (1994): Oxidation of low-density lipoprotein by hypochlorite causes aggregation that is mediated by modification of lysine residues rather than lipid oxidation. *Biochemical Journal*, **302**(Pt 1): 297–304.
- IWASAKI M., INOUE M., OTANI T., SASAZUKI S., KURAHASHI N., MIURA T., YAMAMOTO S., TSUGANE S.

- (2008): Plasma isoflavone level and subsequent risk of breast cancer among Japanese women: a nested case-control study from the Japan Public Health Center-based Prospective Study Group. *Journal of Clinical Oncology*, **26**: 1677–1683.
- JEFREMOV V., ZILMER M., ZILMER K., BOGDANOVIC N., KARELSON E. (2007): Antioxidative effects of plant polyphenols: from protection of G protein signaling to prevention of age-related pathologies. *Annals of the New York Academy of Sciences*, **1095**: 449–457.
- KUO S.M., LEAVITT P.S., LIN C.P. (1998): Dietary flavonoids interact with trace metals and affect metallothionein level in human intestinal cells. *Biological Trace Element Research*, **62**: 135–153.
- KURAHASHI N., IWASAKI M., SASAZUKI S., OTANI T., INOUE M., TSUGANE S. (2007): Soy product and isoflavone consumption in relation to prostate cancer in Japanese men. *Cancer Epidemiology, Biomarkers & Prevention*, **16**: 538–545.
- LIU R.H. (2004): Potential synergy of phytochemicals in cancer prevention: mechanism of action. *Journal of Nutrition*, **134**: 3479S–3485S.
- LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J. (1951): Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, **193**: 265–275.
- MAHN K., BORRÁS C., KNOCK G.A., TAYLOR P., KHAN I.Y., SUGDEN D., POSTON L., WARD J.P., SHARPE R.M., VIÑA J., AARONSON P.I., MANN G.E. (2005): Dietary soy isoflavone-induced increases in antioxidant and eNOS gene expression lead to improved endothelial function and reduced blood pressure *in vivo*. *FASEB Journal*, 1755–1757.
- MANACH C., SCALBERT A., MORAND C., REMESY C., JIMENEZ L. (2004): Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, **79**: 727–747.
- MANACH C., WILLIAMSON G., MORAND C., SCALBERT A., REMESY C. (2005): Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition*, **81**: 230s–242s.
- MILDE J., ELSTNER E.F., GRASSMANN J. (2007): Synergistic effects of phenolics and carotenoids on human low-density lipoprotein oxidation. *Molecular Nutrition & Food Research*, **51**: 956–961.
- OMONI A.O., ALUKO R.E. (2005): Soybean foods and their benefits: potential mechanisms of action. *Nutrition Reviews*, **63**: 272–283.
- PIETTA P.G. (2000): Flavonoids as antioxidants. *Journal of Natural Products*, **63**: 1035–1042.
- RUFER C.E., KULLING S.E. (2006): Antioxidant activity of isoflavones and their major metabolites using different *in vitro* assays. *Journal of Agricultural and Food Chemistry*, **54**: 2926–2931.
- SEVANIAN A., URSINI F. (2000): Lipid peroxidation in membranes and low-density lipoproteins: similarities and differences. *Free Radical Biology & Medicine*, **29**: 306–311.
- VATTEM D.A., GHAEDIAN R., SHETTY K. (2005): Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry. *Asia Pacific Journal of Clinical Nutrition*, **14**: 120–130.
- VAYA J., MAHMOOD S., GOLDBLUM A., AVIRAM M., VOLKOVA N., SHAALAN A., MUSA R., TAMIR S. (2003): Inhibition of LDL oxidation by flavonoids in relation to their structure and calculated enthalpy. *Phytochemistry*, **62**: 89–99.
- YEN G.-C., CHEN H.-Y. (1995): Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry*, **43**: 27–32.
- YEOMANS V.C., LINSEISEN J., WOLFRAM G. (2005): Interactive effects of polyphenols, tocopherol and ascorbic acid on the Cu<sup>2+</sup>-mediated oxidative modification of human low density lipoproteins. *European Journal of Nutrition*, **44**: 422–428.

Received for publication May 13, 2009  
Accepted after corrections March 4, 2010

---

*Corresponding author:*

Dr SHIAN YIN, Chinese Center for Disease Control and Prevention, National Institute for Nutrition and Food Safety, 29 Nan Wei Road, Beijing, 100050, P. R. China  
tel: + 86 10 831 329 32, e-mail: shianyin@gmail.com

---