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Contribution of Tomato Phenolics to Antioxidation and Down-regulation of Blood Lipids

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This study was performed to understand the characteristics and biological activities of phenolics in tomatoes and to examine the effect of tomato on the regulation of blood lipids. Tomatoes of both big and small sizes were used fresh, after blanching, or after blanching and heating. Moreover, a human clinical trial was conducted to examine plasma antioxidation, status of blood lipids, and phenolic responses after ingestion of fresh tomato, tomato juice, and a lycopene drink. The contents of tomato phenolics were increased by 34% for small tomato and by 23% for big tomato after treatment by blanching and heating at 100 °C for 30 min. Tomato phenolics showed fair antioxidant activity (57−71%) and also synergistically promoted the antioxidation (81−100%) of tomato carotenoids. In the human clinical study, total antioxidant capacity and phenolic contents in plasma were increased after administration of fresh tomato and tomato juice, but no significant difference was found for lycopene drink consumption. Triglyceride levels and low-density lipoprotein cholesterol were decreased after administration of fresh tomato and tomato juice, and high-density lipoprotein cholesterol was increased.

KEYWORDS: Tomato; phenolics; lycopene; antioxidation; blood lipids

INTRODUCTION

Many studies show that increased consumption of tomato and tomato-based foods is associated with a lower risk of some diseases (e.g., cancer and cardiovascular disease) (*1*−*4*). It is generally assumed that natural substances (ascorbic acid, tocopherol, and phytochemicals) in tomato account for the beneficial effects. Although tomatoes contain an array of phytochemicals, most attention has been focused on lycopene, thought to be the main contributor to health promotion in human (*5*). However, it is worth investigating whether lycopene is the only contributory substance of tomato or whether any other substances in tomatoes show additive or synergistic effect for lycopene.

Dietary polyphenols may represent an important exogenous defense against oxidative stress (*6*, *7*). Flavonoids are secondary plant metabolites derived from the phenylpropanoid biochemical pathway. Their basic structure consists of two aromatic benzene rings separated by an oxygenated heterocyclic ring (*8*). Flavonoids including flavones, flavonols, flavonones, isoflavones, flavan-3-ols, and anthocyanins are found in plant tissues (*9*). Over 4000 naturally occurring flavonoids have been described; most of them conjugate to sugar molecules and are commonly located in the upper epidermal layer of leaves. The endogenous flavonols are present in tomato tissues primarily as conjugates. Tomatoes and related products contain rich conjugated quercetin and kaempherol (*10*).

Plasma lipid and its metabolism are well-established risk factors of coronary artery disease (CAD). Increased risk of CAD has been associated with elevated triglycerides, total cholesterol, and low-density lipoprotein cholesterol (LDL-C) and with decreased high-density lipoprotein cholesterol (HDL-C) (*11*−*14*). It was reported that phenolic compounds were associated with antioxidant activity and played an important role in stabilizing lipid peroxidation (*15*, *16*). Flavonoids are very efficient scavengers of peroxyl radicals, and the action could be related to their capacity to reduce and chelate ferric iron, which catalyzes lipid peroxidation (*17*).

This study examined characteristics of phenolics in small (*Lycopersicon esculentum* Mill cv. Santa) and big tomatoes (*L. esculentum* Mill cv. Farmers301), both fresh and after heat treatments. In addition, the antioxidant activity of tomato phenolics and its effect on carotenoids were also determined in this study. In a human clinical trial, plasma antioxidation, blood lipid status, and phenolic levels after ingestion of fresh tomato, tomato juice, or a lycopene drink continuously for 6 weeks were evaluated, respectively.

MATERIALS AND METHODS

Sample Preparation. Small tomatoes (*L. esculentum* Mill cv. Santa) and big tomatoes (*L. esculentum* Mill cv. Farmers301) were purchased from a village farm. Fresh, blanched (in 85 °C water for 6 and 30 min, respectively), and blanched and then heated (separately in boiling water for 10 and 30 min, respectively) tomatoes were used in this study. For the human clinical trial, tomato juice and food-grade lycopene were kindly provided by AGV Products Corp., Taiwan. Tomato juice was produced through the following procedure. Fresh tomatoes were washed

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and then blended. The tomato seeds were removed. Residual juice was blanched at 85 °C for 6 min and further sterilized at 100 °C for 10 min. Each subject took a daily serving of fresh small tomatoes (500 g), tomato juice (600 mL), or lycopene drink (600 mL). The above tomato samples contained the same amount of lycopene (40 mg), and 186.18, 226.11, and 0 mg of phenolics, respectively. The lycopene contents in fresh tomato were well correlated with red color and weight. To get the constant levels of lycopene, fresh small tomatoes with constant colorimetric properties (a^*/b^* Hunter values = 1.34 ± 0.25) were used according to the method of Lavelli et al. (*18*). The lycopene drink was prepared by adding 40 mg of lycopene and 2.5 g of sugar in 600 mL of water; lycopene was dispersed in water by homogenization.

Crude Phenolic Extract. Crude phenolic extract of tomato was prepared according to our previous study (*19*). In brief, the sample was extracted with 80% aqueous acetone (1:1, w/v, Merck, Darmstadt, Germany) in a Waring blender (Osterizer, Mexico) for 1 min, followed by soaking for 5 min before filtration, and finally filtered through no. 4 Whatman paper on a Büchner funnel under vacuum. The filtrate was evaporated by a rotary evaporator (Eyela, Tokyo, Japan) under vacuum condition. The dried residual material, crude phenolic extract, was frozen at −20 °C before use.

Crude Carotenoid Extract. Crude carotenoid extract of tomato was obtained according to the method of Abushita et al. (*20*). In brief, the sample was extracted with methanol (1:1, w/v, Merck) in a Waring blender for 1 min. The mixture was mixed with 3 volumes of chloroform and mechanically shaken for 20 min, followed by soaking for 5 min before filtration, and finally filtered through no. 4 Whatman paper on a Büchner funnel under vacuum. The filtrate was evaporated by a rotary evaporator under vacuum condition. The dried material, crude carotenoid extract, was also frozen at −20 °C before use.

Estimation of Total Phenolic Content. Total phenolic contents were determined using Folin−Ciocalteu phenol reagent (Merck) (*21*). A sample of 100 μL was diluted with water to 2 mL in a 10 mL measuring flask. Then, 1 mL of Folin−Ciocalteu phenol reagent was added, and the flask was vigorously shaken. Immediately, 5 mL of 2.4 mol/L sodium carbonate solution (Wako, Saitama, Japan) was pipetted, followed by thorough shaking. After 20 min, the absorbance of the mixture was read at 735 nm (spectrophotometer, U-2001, Hitachi, Japan) without background measurements and using gallic acid (Sigma-Aldrich, St. Louis, MO) as a standard. Total phenolic contents were expressed as micrograms of gallic acid equivalent per gram of fresh weight.

Analysis of Phenolics by High-Performance Liquid Chromatography. Crude phenolic extracts were analyzed according to the methods of Hertog et al. and Schieber et al. (*22*, *23*). Standards of phenolic compounds including gallic acid, chlorogenic acid, caffeic acid, rutin, coumuric acid, sinapic acid, naringin, myricetin, quercetin, cinnamic acid, naringenin, and kaempferol were purchased from Sigma-Aldrich. The separation of phenolic compounds was performed by HPLC equipped with chromatography data station software (Hitachi model D-6500), a binary gradient intelligent pump (L-6200A, Hitachi), and a diode array detection system (L4500A, Hitachi). A Lichrospher 100 RP-18e (Merck) column (5 μm, 25 cm × 4 mm i.d.) and a security guard RP-18e (4 × 3.0 μm i.d.) were used. The mobile phase consisted of 2.5% (v/v) acetic acid in water (eluent A) and of acetonitrile (eluent B). The gradient program consisted of 3% B initially, changed to 21% B in 4 min, maintained for 6 min, increased to 22% B in 11 min, held for 4 min, changed to 35% B in 16 min, held for 19 min, increased to 100% B in 36 min, held for 4 min, and returned to 3% B in 45 min. The injection volume for all samples was 20 μL. Simultaneous monitoring was set at 266 nm. The flow rate was 0.8 mL/min. Spectra were recorded from 200 to 400 nm.

Antioxidant Activity (AOA). Antioxidant activity was determined according to linoleic acid oxidation (*24*). AOA was expressed as the following: $AOA = (A_{234(c)} - A_{234})/A_{234(c)}$, where A_{234} is the difference in absorbance in the presence of sample between the beginning and end of a 15 h incubation at 37 °C at 234 nm, and $A_{234(c)}$ is the difference in absorbance in the absence of sample between the beginning and the end of a 15 h incubation at 37 °C at 234 nm.

Metal Ion Chelating Assay. The ferrous ion chelating potential was determined according to the method of Decker and Welch (*25*), wherein the Fe^{2+}-chelating ability was monitored by absorbance of the ferrous iron−ferrozine complex at 562 nm. Briefly, the reaction mixture, containing 300 μL samples of different concentrations, 1.1 mL of methanol (Merck), 30 μL of $FeCl_2$ (2 mmol/L, Sigma-Aldrich), and 60 μL of ferrozine (5 mmol/L), was shaken well and incubated for 10 min at room temperature. The absorbance of the mixture was measured at 562 nm. The ability to chelate ferrous ion was calculated using the following equation: chelating effect (%) = $(A_{562,sample}/A_{562,control}) \times 100\%$.

Table 1. Anthropometric Characteristics of Enrolled Subjects[a]

	fresh tomato ($n = 8$)	tomato juice ($n = 8$)	lycopene drink ($n = 8$)
age (years)	18–23	19–22	18–22
height (cm)	163.00 ± 11.25	166.13 ± 11.51	166.25 ± 10.90
weight (kg)	57.19 ± 12.33	54.80 ± 5.84	55.45 ± 8.12
body fat (%)	22.25 ± 5.90	21.38 ± 6.55	21.63 ± 5.97
BMI[b] (kg/m^2)	21.32 ± 2.36	19.89 ± 1.49	19.97 ± 1.01

[a] Values are expressed as mean ± SD. [b] BMI, body mass index.

Human Clinical Trial. A total of 24 healthy participants were recruited via advertisements and randomly divided into three groups ($n = 8$ for each group). Informed written consent was obtained from all volunteers. This study was approved by the Institutional Review Board Committee of Chung Shan Medical University Hospital. Moreover, all participants were between 18 and 23 years old and did not use any medications or supplements (e.g., vitamins) during the period of this study. Characteristics of subjects enrolled are given in **Table 1**. They could withdraw from the study at any time if desired. All diets during the study period were supplied and designed by registered dietitians. According to the dietary recommendation, 2450 and 2000 kcal/day diets were given for each man and woman, respectively. Every day, 40 mg of lycopene (in 500 g of fresh tomato, 600 mL of tomato juice, and 600 mL of a lycopene drink, respectively) was served as a part of the meal at lunch and dinner (*26*). No other foods containing lycopene were allowed during the experimental period. The total duration administration of this study was 6 weeks, and another 2 weeks were also included as follow-up monitoring. Fasting blood samples were obtained at the beginning, at the third and sixth weeks during the experimental period, and at the end of the follow-up period.

Thiobarbituric Acid-Reactive Substances (TBARS) of Plasma. The total TBARS in plasma was determined by reacting with 2-thiobarbituric acid (TBA, Sigma-Aldrich) at 90−100 °C (*27*). Malondialdehyde (MDA) or MDA-like substances and TBA react together to produce a pink pigment having an absorbance at 532 nm (spectrophotometer, U-2001, Hitachi). The reaction was performed at pH 2−3 at 90 °C for 15 min. The sample was mixed with 2 volumes of cold 0.7 mol/L trichloroacetic acid (TCA, Merck) to precipitate protein. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 46.8 mmol/L TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm and using 1,1,3,3-tetramethoxypropane (Sigma-Aldrich) as standard. TBARS value was expressed as micromoles of MDA per liter of plasma.

Estimation of Phenolic Content in Plasma. Total phenolic contents in plasma were measured by the Folin−Ciocalteu method as the above description (*28*). The phenolic compounds were determined after a procedure of extraction/hydrolysis and protein precipitation with 30% metaphosphoric acid (MPA; Wako, Saitama, Japan). To hydrolyze the conjugated polyphenols, 0.5 mL of 1 mol/L HCl was added to 100 μL of the sample, followed by 1 min of vortexing and incubation at 37 °C for 30 min. Then, 0.5 mL of 2 mol/L NaOH (Wako) in 75% methanol (Merck) was added, and the resulting mixture was incubated at 37 °C for 30 min. This step broke the linkage between polyphenols and lipids and also provided the first extraction of polyphenols. Then, 0.5 mL of 30% MPA was added to remove plasma proteins. After centrifuging at 1500*g* (Supercentrifuge, 1K15, Sigma), the supernatant was assayed for total phenolic contents with Folin−Ciocalteu phenol reagent (Merck). The results are expressed as micrograms of gallic acid equivalent per milliliter of plasma.

Table 2. Contents of Total Phenolics in Tomato[a]

	total phenolic contents (μg/g of fresh wt)[b]		
	big tomato	small tomato	*P* value[c]
fresh	218.86 ± 53.3ab	372.35 ± 19.86b	0.03
blanching	238.97 ± 37.65a	390.64 ± 3.73b	0.01
blanching + 100 °C/ 10 min	259.22 ± 14.07a	397.81 ± 23.64b	0.03
blanching + 100 °C/ 30 min	292.36 ± 25.43a	457.96 ± 32.13a	0.09

[a] Values are expressed as mean ± SD. Data within the same column bearing different letters (a,b) were significantly different ($p < 0.05$). [b] Micrograms of gallic acid equivalent per gram of fresh wt. [c] Student's *t* test was used to assess statistical significance between big and small tomatoes.

Plasma Lipid Analyses. Levels of TC, TG, LDL-C, and HDL-C in plasma were determined with commercially available kits using the biochemical analyzer (ARCO, Italy).

Statistical Analysis. All results are presented as mean ± standard deviation (SD). Differences for the in vitro study were determined by Student's *t* test and one-way ANOVA (SAS for Windows, version 8.02; SAS Institute, Cary, NC) followed by Duncan's post-hoc test for multiple comparisons. Differences for the human clinical trial at the third and sixth weeks and the follow-up (F/U) period were compared with values at baseline (initial) using one-way repeated measures analysis of variance or the Friedman repeated measures analysis of variance on ranks within each group (SigmaState, version 2.03; Jandel Scientific, San Rafael, CA). Significant difference was accepted when $p < 0.05$.

RESULTS AND DISCUSSION

Tomato is consumed worldwide. In Taiwan, small and big tomatoes are often part of the meal. In this study, some heat treatments, including blanching and blanching followed by heating at 100 °C for 10 or 30 min, were used to simulate the actual cooking. Peroxidase was destroyed as the blanching indicator in this study. Heating times of 6 and 30 min at 85 °C were used for small and big tomatoes, respectively. Thermal processing at 85 °C deactivates the oxidative and hydrolytic enzymes to avoid the loss of phenolic acids (*29*).

Phenolic Levels. The total phenolic contents of two commonly consumed fresh tomatoes in Taiwan are given in **Table 2**. Phenolic levels were found to increase after heating, especially by severe heat treatment (blanching and heating at 100 °C for 30 min). Furthermore, small tomatoes had higher levels than big tomatoes. This revealed that heat treatment could cause the release of phenolics (*29*). The amounts of total phenolics determined by the Folin−Ciocalteu assay were considerably similar to those reported in the literature for tomatoes (101−400 μg/g) (*30*, *31*).

Phenolic Profile. Figure 1 shows the HPLC of phenolic compounds. As seen in **Figure 2**, both small and big tomatoes had abundant phenolic constituents. The phenolics isolated from fresh tomato and heat treatment tomato were gallic acid, chlorogenic acid, caffeic acid, myricetin, and naringenin. Big tomato has more myricetin than small tomato. In contrast to a previous study (*10*), high quantities of quercetin and kaempferol were not found in this study. This was probably due to the different tomato varieties. One unknown compound (peak A) with glycoside property, displaying the characteristic UV spectrum, was detected. This compound was decreased after heating, followed by an increase in myricetin (peak 8), and a new compound then appeared by its side (peak B). Furthermore, after acid hydrolysis at pH 3 (90 °C for 2 h), similar phenomena were observed (data not shown). This revealed that heat treatment probably split the sugar moiety from the glycoside.

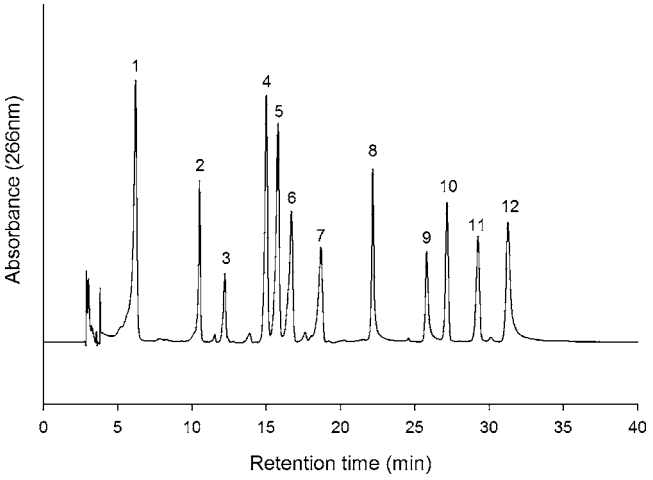

Figure 1. High-performance liquid chromatogram of standard mixture of phenolic compounds: 1, gallic acid; 2, chlorogenic acid; 3, caffeic acid; 4, rutin; 5, coumuric acid; 6, sinapic acid; 7, naringin; 8, myricetin; 9, quercetin; 10, cinnamic acid; 11, naringenin; 12, kaempferol. Detection wavelength was set at 266 nm, and flow rate was 0.8 mL/min.

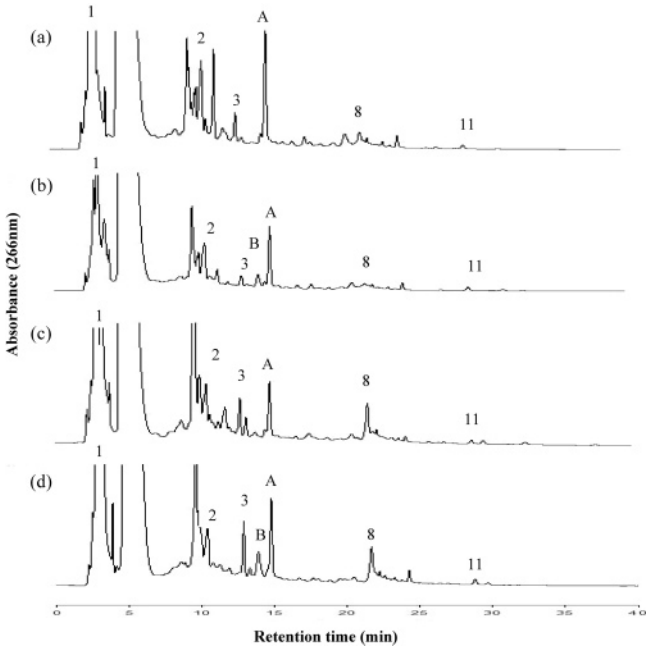

Figure 2. High-performance liquid chromatogram of phenolics in fresh small tomato (**a**), heated (100 °C/30 min) small tomato (**b**), fresh big tomato (**c**), and heated (100 °C/30 min) big tomato (**d**). Detection wavelength was set at 266 nm, and flow rate was 0.8 mL/min. Concentrations of 150 mg/mL for all extract samples were used. Peaks: 1, gallic acid; 2, chlorogenic acid; 3, caffeic acid; A, unknown compound **1**; 8, myricetin; 11, naringenin; B, unknown compound **2**.

Antioxidant Activity. The effects of crude phenolic extracts and crude carotenoid extracts from tomato on the peroxidation of linoleic acid are shown in **Figure 3**. As can be seen, oxidation of linoleic acid was markedly suppressed by all samples in a dose-dependent manner. Both crude phenolic and carotenoid extracts showed better antioxidation than ascorbic acid. Crude phenolic extracts of tomato had fair antioxidant activity either with or without heat treatments. Heat treatment of tomatoes greatly increased the levels of phenolics. However, heating had

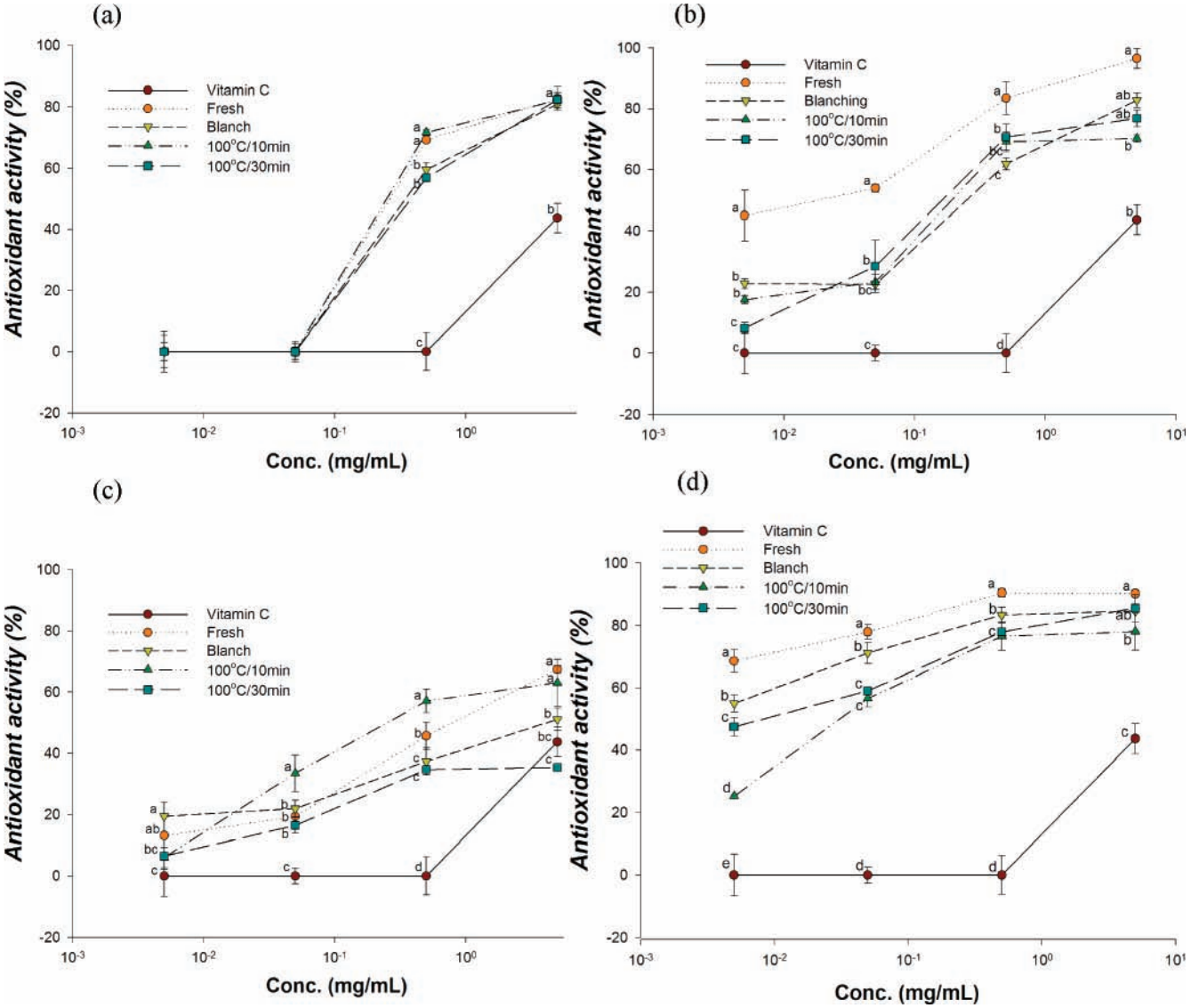

Figure 3. Antioxidant activity of crude phenolic and carotenoid extracts from different heat treatments of tomatoes: crude phenolics of big tomato (**a**); crude carotenoids of big tomato (**b**); crude phenolics of small tomato (**c**); crude carotenoids of small tomato (**d**). Extract concentrations of 0.005, 0.05, 0.5, and 5 mg/mL were used. Each bar represents the mean ± SD. Values at each concentration without a letter in common were significantly different ($p < 0.05$).

no significant effect on antioxidant activity of phenolic extracts when the same concentrations were tested. This is probably due to the release and conversion of phenolics and also the complex reaction of phenolics in tomatoes during heating (e.g., reduction of carotenoids). However, crude carotenoid extracts from fresh tomatoes showed better antioxidant activity than those from heat-treated ones. In addition, crude phenolics showed a synergistic effect on the antioxidation of crude carotenoids (**Table 3**).

Chelating Activity. The chelating effects of all crude phenolic extracts and crude carotenoid extracts from tomato on ferrous ion are shown in **Figure 4**. As can be seen, crude carotenoid extracts displayed weak function, whereas crude phenolic extracts showed perfect chelating ability on ferrous ions in a dose-dependent manner. The chelating function of phenolics is probably contributed by the flavonoids, in which 3′,4′-dihydroxy and 3-hydroxy-4-carbonyl at position of rings B and C work, respectively (*32*).

Human Clinical Trial. Clinical evaluation showed that plasma phenolics were significantly increased after the consumption of fresh tomato and tomato juice for 3 weeks (**Table 4**), but no difference was found for lycopene drink administration. Higher antioxidation was found for both fresh tomato and tomato juice administrations, but not for lycopene drink

Table 3. Antioxidant Activity of Phenolic and Carotenoid Extracts and Their Mixture[a]

	antioxidant activity (%)	
	phenolic extract	carotenoid extract
fresh		
carotenoid extracts		83.49
phenolic extracts	69.2	94.78[b]
blanching		
carotenoid extracts		61.93
phenolic extracts	59.48	87.2[b]
blanching +100 °C/10 min		
carotenoid extracts		69.18
phenolic extracts	71.63	81.24[b]
blanching +100 °C/30 min		
carotenoid extracts		76.08
phenolic extracts	56.87	100[b]

[a] 0.5 mg/mL for phenolic extract or carotenoid extract, respectively. [b] Mixture of 0.25 mg of phenolic extract and 0.25 mg of carotenoid extract.

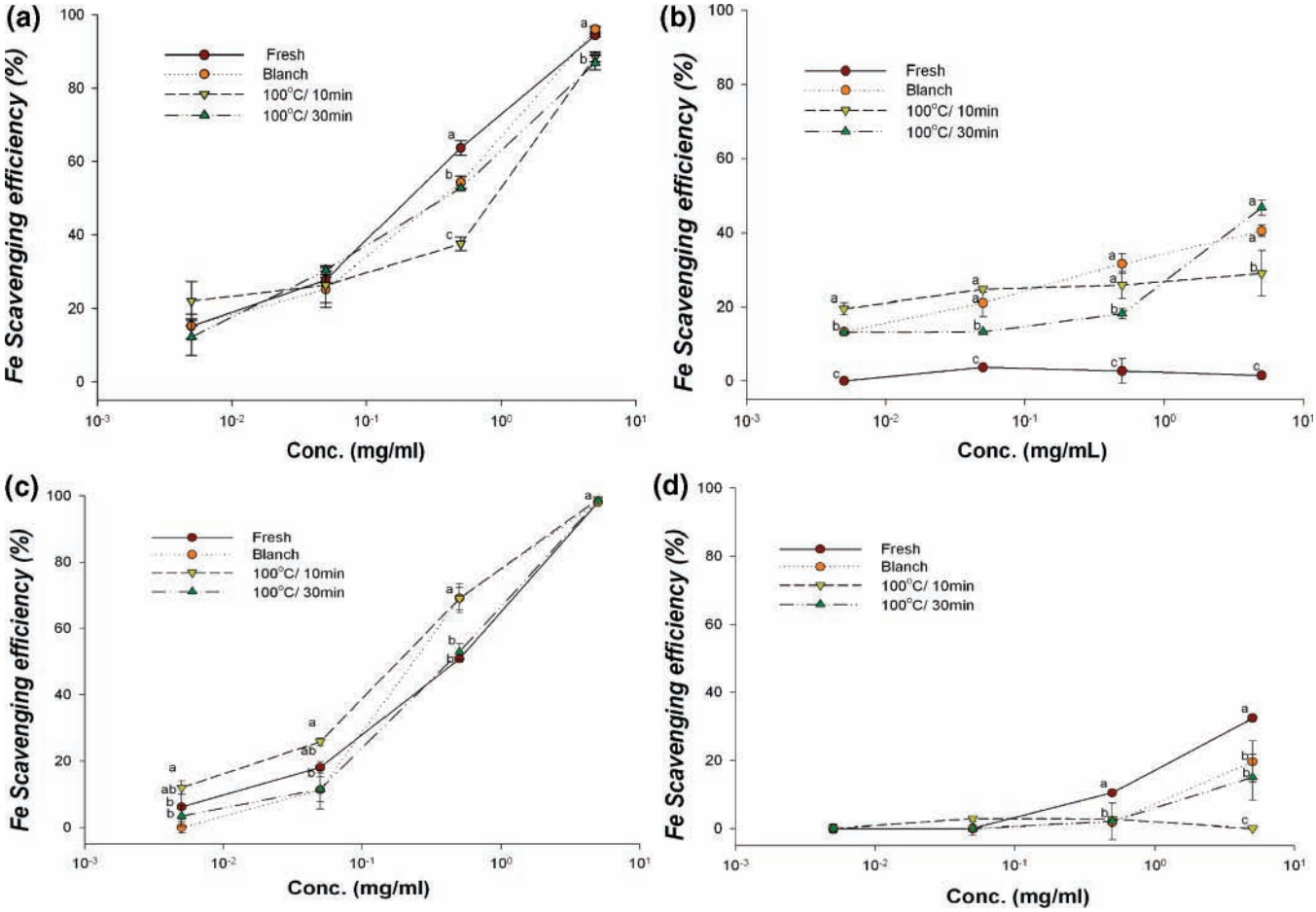

Figure 4. Chelating activity of crude phenolic and carotenoid extracts from different heat treatments of tomatoess: crude phenolics of big tomato (**a**); crude carotenoids of big tomato (**b**); crude phenolics of small tomato (**c**); crude carotenoids of small tomato (**d**). Extract concentrations of 0.005, 0.05, 0.5, and 5 mg/mL were used. Each bar represents the mean ± SD. Values at each concentration without a letter in common were significantly different ($p < 0.05$).

Table 4. Phenolic Contents, Antioxidation, and TBARS of Enrolled Subjects[a]

	initial	third week	sixth week	F/U period[b]
		Antioxidant Capacity (%)		
fresh tomato	73.90 ± 5.14	78.31 ± 4.40	80.79 ± 5.12	72.96 ± 2.09
tomato juice	72.48 ± 3.74	74.69 ± 2.61	76.86 ± 4.13*	71.03 ± 3.75
lycopene drink	73.16 ± 2.90	72.65 ± 1.58	72.17 ± 2.60	72.91 ± 3.38
		TBARS[c]		
fresh tomato	3.96 ± 1.51	5.84 ± 1.39	4.50 ± 2.15	5.45 ± 0.86
tomato juice	3.43 ± 1.76	4.63 ± 1.12	3.83 ± 2.14	5.32 ± 1.28
lycopene drink	5.70 ± 1.31	4.39 ± 1.55	5.51 ± 0.95	5.70 ± 0.97
		Phenolic Contents[d]		
fresh tomato	585.35 ± 113.63	971.89 ± 178.81*	709.30 ± 129.42	562.64 ± 116.9
tomato juice	604.82 ± 190.65	972.48 ± 133.81*	976.89 ± 146.89*	683.29 ± 44.38
lycopene drink	758.45 ± 216.10	772.61 ± 217.18	696.41 ± 179.01	781.59 ± 75.35

[a] Values are expressed as mean ± SD. *, significant difference as compared with the initial ($p < 0.05$). [b] F/U period, follow-up period. [c] TBARS, thiobarbituric acid-reactive substances, μmol of MDA/L of plasma. [d] μg of gallic acid equivalent/mL of plasma.

Table 5. Blood Lipids Status of Enrolled Subjects[a]

	initial	third week	sixth week	F/U period[b]
		Total Cholesterol (mg/dL)		
fresh tomato	189.00 ± 26.11	184.50 ± 22.39	173.67 ± 13.00	186.00 ± 24.62
tomato juice	199.00 ± 38.62	185.00 ± 31.92	183.67 ± 30.45	203.83 ± 31.75
lycopene drink	195.88 ± 29.71	193.00 ± 30.76	200.50 ± 30.35	201.75 ± 34.32
		Triglyceride (mg/dL)		
fresh tomato	86.71 ± 40.73	65.66 ± 31.19	43.00 ± 11.08*	71.46 ± 23.04
tomato juice	80.76 ± 31.88	52.90 ± 13.97*	54.33 ± 19.29*	78.34 ± 41.82
lycopene drink	62.82 ± 27.26	54.33 ± 21.84	58.03 ± 20.08	65.9 ± 21.17
		HDL-C[c] (mg/dL)		
fresh tomato	39.43 ± 7.21	42.78 ± 7.85	46.83 ± 7.53*	45.06 ± 4.82*
tomato juice	45.16 ± 3.82	46.43 ± 4.60	50.84 ± 4.96*	46.17 ± 4.50
lycopene drink	47.37 ± 9.84	48.66 ± 10.48	47.28 ± 9.02	46.35 ± 7.02
		LDL-C[d] (mg/dL)		
fresh tomato	116.25 ± 14.44	113.75 ± 16.30	101.96 ± 14.38	115.75 ± 16.16
tomato juice	113.00 ± 13.81	116.00 ± 9.13	103.75 ± 10.27*	116.00 ± 9.13
lycopene drink	112.00 ± 10.81	112.65 ± 13.86	109.32 ± 13.21	114.33 ± 12.16

[a] Values are expressed as mean ± SD. *, significant difference as compared with the initial ($p < 0.05$). [b] F/U period, follow-up period. [c] HDL-C,high-density lipoprotein cholesterol, [d] LDL-C, low-density lipoprotein cholesterol.

consumption. All measurements for TBARS showed no difference as compared with the initial period, which was probably because all subjects were healthy. As for the blood lipid status, triglyceride (TG) levels were greatly decreased after 6 weeks of fresh tomato administration and after 3 weeks of tomato juice administration. HDL-C levels were increased after the consumption of fresh tomato and tomato juice for 6 weeks (**Table 5**). In addition, LDL-C levels were decreased after 6 weeks of tomato juice consumption. The other human studies also showed increasing HDL-C (*33*) levels and decreasing TG (*34*) and LDL-C (*35*) levels after the consumption of a tomato-rich diet. Subjects consuming tomato juice had a better effect on lipid regulation than did those consuming fresh tomato, probably due to the greater phenolic content of fresh tomato. These results

indicated that tomato phenolics probably play an important role in blood lipid regulation and CAD prevention.

To understand which kind of processed tomatoes can benefit human healt, fresh small tomato and tomato juice were used in this human clinical trial. Moreover, different studies indicate that lipid regulation by tomato and tomato-related products was accredited to lycopene (*35*), which probably increased the availability of acetyl CoA, a positive regulator of HMG CoA reductase, or enhanced LDL degradation and plaque rupture. However, this study showed that blood lipid levels (TG, HDL-C, and LDL-C) were altered by the administration of fresh tomato and tomato juice, but no difference was found for lycopene drink consumption. This result suggested that fresh and cooked tomato could down-regulate blood lipid, but lycopene drink alone could not. Perhaps not only lycopene could contribute to better health, but phenolics also played an important role.

The human clinical test of the current study had some limitation. As mentioned, this experimental design using food grade lycopene as control to differentiate the effect of lycopene from that of the phenolics in the CAD impact is difficult to define because the bioavailabilities of the lycopene, comparing the lycopene naturally occurring in the fresh and processed tomatoes and the same compound found in the food grade extract dissolved in water, are very different. In addition, plasma phenolic content in the fresh tomato group at 6 weeks is smaller than that at 3 weeks. Why did long-term tomato consumption lead to such a phenomenon? Is it from human body regulation? Further investigation will be required. Finally, the TBARS method used in the study may have sufficient power to detect converts. However, individual difference probably leads to such result.

In conclusion, tomato phenolics were greatly released after heating, especially by severe heating (blanching and heating at 100 °C for 30 min). Tomato phenolics had fair antioxidant activity and synergistically promoted the antioxidation of lycopene. Clinical evaluation showed that plasma antioxidation and phenolic contents were increased after administration of fresh tomato and tomato juice, but no difference was found for lycopene drink consumption. TG levels were decreased after administration of fresh tomato and tomato juice. HDL-C was found to increase whereas LDL-C was found to decrease after the consumption of fresh tomato and tomato juice. This clearly revealed that tomato phenolics probably played an important role in health promotion. Determining the function of tomato phenolics on human health merits further exploration.

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LITERATURE CITED

(1) Das, S.; Otani, H.; Maulik, N.; Das, D. K. Lycopene, tomatoes, and coronary heart disease. *Free Radical Res.* **2005**, *39*(4), 449−455.

(2) Blum, A.; Monir, M.; Wirsansky, I.; Ben-Arzi, S. The beneficial effects of tomatoes. *Eur. J. Intern. Med.* **2005**, *16* (6), 402−404.

(3) Wolk, A. Diet, lifestyle and risk of prostate cancer. *Acta Oncol.* **2005**, *44* (3), 277−281.

(4) Nkondjock, A.; Ghadirian, P.; Johnson, K. C.; Krewski, D. Dietary intake of lycopene is associated with reduced pancreatic cancer risk. *J. Nutr.* **2005**, *135* (3), 592−597.

(5) Di Mascio, P.; Kaiser, S.; Sies, H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.* **1989**, *274* (2), 532−538.

(6) Pandjaitan, N.; Howard, L. R.; Morelock, T.; Gil, M. I. Antioxidant capacity and phenolic content of spinach as affected by genetics and maturation. *J. Agric. Food Chem.* **2005**, *53*, 8618−8623.

(7) Yu, J.; Wang, L.; Walzem, R. L.; Miller, E. G.; Pike, L. M.; Patil, B. S. Antioxidant activity of citrus limonoids, flavonoids, and coumarins. *J. Agric. Food Chem.* **2005**, *53*, 2009−2014.

(8) Torres, C. A.; Davies, N. M.; Yanez, J. A.; Andrews, P. K. Disposition of selected flavonoids in fruit tissues of various tomato (*Lycopersicon esculentum* Mill.) genotypes. *J. Agric. Food Chem.* **2005**, *53*, 9536−9543.

(9) Haslam, E. *Practical Polyphenolics—from Structure to Molecular Recognition and Physiological Action*; Cambridge University Press: Cambridge, U.K., 1998.

(10) Stewart, A. J.; Bozonnet, S.; Mullen, W.; Jenkins, G. I.; Lean, M. E.; Crozier, A. Occurrence of flavonols in tomatoes and tomato-based products. *J. Agric. Food Chem.* **2000**, *48*, 2663−2669.

(11) Austin, M. A.; Hokanson, J. E.; Edwards, K. L. Hypertriglyceridemia as a cardiovascular risk factor. *Am. J. Cardiol.* **1998**, *81* (4A), 7B−12B.

(12) Castelli, W. P.; Anderson, K.; Wilson, P. W.; Levy, D. Lipids and risk of coronary heart disease. The Framingham study. *Ann. Epidemiol.* **1992**, *2* (1−2), 23−28.

(13) Davignon, J.; Cohn, J. S. Triglycerides: a risk factor for coronary heart disease. *Atherosclerosis* **1996**, 124 (Suppl.), S57−S64.

(14) Gordon, D. J.; Rifkind, B. M. High-density lipoprotein—the clinical implications of recent studies. *N. Engl. J. Med.* **1989**, *321* (19), 1311−1316.

(15) Hakimoglu, F.; Kizil, G.; Kanay, Z.; Kizil, M.; Isi, H. The effect of ethanol extract of *Hypericum lysimachioides* on lipid profile in hypercholesterolemic rabbits and its in vitro antioxidant activity. *Atherosclerosis* **2007**, *192* (1), 113−122.

(16) Sriplang, K.; Adisakwattana, S.; Rungsipipat, A.; Yibchok-Anun, S. Effects of *Orthosiphon stamineus* aqueous extract on plasma glucose concentration and lipid profile in normal and streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* **2007**, *109* (3), 510−514.

(17) Martínez-Valverde, I.; Periago, M. J.; Provan, G.; Chesson, A. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). *J. Sci. Food Agric.* **2002**, *82*, 323−330.

(18) Lavelli, V.; Hippeli, S.; Peri, C.; Elstner, E. F. Evaluation of radical scavenging activity of fresh and air-dried tomatoes by three model reactions. *J. Agric. Food Chem.* **1999**, *47*, 3826−3831.

(19) Wang, C. K.; Hwang, L. S. Analysis of the phenolic compounds in betel quid. *J. Chin. Agric. Chem. Soc.* **1993**, *31*, 623−632.

(20) Abushita, A. A.; Hebshi, E. A.; Daood, H. G.; Biacs, P. A. Determination of antioxidant vitamins in tomatoes. *Food Chem.* **1997**, *60*, 207−212.

(21) Julkunen-Tiitto, R. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *J. Agric. Food Chem.* **1985**, *33*, 213−217.

(22) Hertog, M. G. L.; Hollman, P. C. H.; Venema, D. P. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *J. Agric. Food Chem.* **1992**, *40*, 1591−1598.

(23) Schieber, A.; Keller, P.; Carle, R. Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. *J. Chromatogr., A* **2001**, *910* (2), 265−273.

(24) Wang, C. K.; Lee, W. H. Separation, characteristics, and biological activities of phenolics in areca fruit. *J. Agric. Food Chem.* **1996**, *44*, 2014−2019.

(25) Dinis, T. C.; Maderia, V. M.; Almeida, L. M. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.* **1994**, *315* (1), 161−169.

(26) Pool-Zobel, B. L.; Bub, A.; Muller, H.; Wollowski, I.; Rechkemmer, G. Consumption of vegetables reduces genetic damage in humans: first results of a human intervention trial with carotenoid-rich foods. *Carcinogenesis* **1997**, *18* (9), 1847−1850.

(27) Sogut, S.; Zoroglu, S. S.; Ozyurt, H.; Yilmaz, H. R.; Ozugurlu, F.; Sivasli, E.; Yetkin, O.; Yanik, M.; Tutkun, H.; Savas, H. A.; Tarakcioglu, M.; Akyol, O. Changes in nitric oxide levels and antioxidant enzyme activities may have a role in the pathophysiological mechanisms involved in autism. *Clin. Chim. Acta* **2003**, *331* (1−2), 111−117.

(28) Serafini, M.; Maiani, G.; Ferro-Luzzi, A. Alcohol-free red wine enhances plasma antioxidant capacity in humans. *J. Nutr.* **1998**, *128* (6), 1003−1007.

(29) Dewanto, V.; Wu, X.; Adom, K. K.; Liu, R. H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010−3014.

(30) George, B.; Kaur, C.; Khurdiya, D. S.; Kapoor, H. C. Antioxidants in tomato (*Lycopersicon esculentum*) as a function of genotype. *Food Chem.* **2004**, *84*, 45−51.

(31) Huang, H. Y.; Chang, C. K.; Tso, T. K.; Huang, J. J.; Chang, W. W.; Tsai, Y. C. Antioxidant activities of various fruits and vegetables produced in Taiwan. *Int. J. Food Sci. Nutr.* **2004**, *55* (5), 423−429.

(32) Sugihara, N.; Arakawa, T.; Ohnishi, M.; Furuno, K. Anti- and pro-oxidative effects of flavonoids on metal-induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with α-linolenic acid. *Free Radical Biol. Med.* **1999**, *27* (11−12), 1313−1323.

(33) Blum, A.; Merei, M.; Karem, A.; Blum, N.; Ben-Arzi, S.; Wirsansky, I.; Khazim, K. Effects of tomatoes on the lipid profile. *Clin. Invest. Med.* **2006**, *29* (5), 298−300.

(34) Ahuja, K. D.; Pittaway, J. K.; Ball, M. J. Effects of olive oil and tomato lycopene combination on serum lycopene, lipid profile, and lipid oxidation. *Nutrition* **2006**, *22* (3), 259−265.

(35) Fuhrman, B.; Elis, A.; Aviram, M. Hypocholesterolemic effect of lycopene and β-carotene is related to suppression of cholesterol synthesis and augmentation of LDL receptor activity in macrophages. *Biochem. Biophys. Res. Commun.* **1997**, *233* (3), 658−662.

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