The Antioxidant Properties of Lycopene Concentrate Extracted from Tomato Paste

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ABSTRACT: Lycopene concentrate (LC) containing 50 wt% lycopene was extracted from tomato paste. The antioxidant properties of LC were evaluated by means of chemiluminescence in four models. The four models were superoxide anions generated from pyrogallol autoxidation, hydroxyl radicals from Fenton reaction, singlet oxygens from OH^--NaClO-H_2O_2, and lipid peroxidation from 2,2′-azobis(2-amidinopropane)dihydrochloride-induced γ-linolenic acid. LC was an effective scavenger toward superoxide anions, hydroxyl radicals, and singlet oxygens, and also it could effectively reduce lipid peroxidation. The 50% efficient concentrations (EC_{50}) toward superoxide anions, hydroxyl radicals, lipid peroxidation and singlet oxygen were 0.75, 0.05, 0.1, and 1 mg/mL, respectively. In addition, changes of antioxidant behaviors with time were investigated. The time requirements of LC for effectively scavenging superoxide anions, hydroxyl radicals, and inhibiting lipid peroxidation were not higher than 6, 6, and 18 s, respectively.

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KEY WORDS: Antioxidant, chemiluminescence, free radicals, lycopene.

Many reports from the epidemiologic literature infer that high intakes of tomato and tomato products are beneficial to health (1). The benefits of such foods are attributed to their antioxidant properties, especially to the antioxidant properties of lycopene contained therein. In fact, antioxidant properties of lycopene have been investigated extensively as a potential protective agent against the cancers of the prostate, cervix, colon, breast, and other chronic diseases (2).

The antioxidant properties of lycopene are commonly evaluated in two ways. One is through examining its protective effect against oxidative damage to biological molecules like DNA, lipids, and protein in cell culture or animal experiments. For example, lycopene has been reported to be effective in prevention of oxidative damage to lymphocyte DNA (3), to cell membrane damage (4), and to HT29 cell. (5) The other way is through measuring its capability to scavenge free radicals directly. Lycopene is a most effective singlet oxygen quencher (6), and direct reactions between lycopene and the radicals of nitrogen dioxide (NO_2), thiol (RS), and sulfonyl (RSO_2) (7) have also been investigated. However, the direct reaction between lycopene and reactive oxygen species (ROS) like superoxide anions, hydroxy radical, and the like is rarely reported.

Here, a concentrate containing a high level of lycopene (LC) was extracted from tomato paste. LC was expected to be used as anti-cancer medicines or healthcare products. The purpose of this study was to evaluate the antioxidant properties of LC toward superoxide anions, hydroxyl radicals, lipid peroxidation, and singlet oxygens by using a chemiluminescence technique.

EXPERIMENTAL PROCEDURES

LC used here was extracted from tomato paste. The extraction conditions were as follows: tomato paste was pretreated using 95% alcohol at 50°C to get rid of water-soluble substances. Then, tomato paste was extracted using hexane at 50°C. After extraction, hexane was evaporated to dryness under a stream of nitrogen with a vacuum evaporator of Shanghai No. 6 Instrument Factory (Shanghai, China). The residue remaining was a lycopene concentrate, in which lycopene content was 50%, as assessed by a λ-20 type ultraviolet (UV) spectrometer (PerkinElmer, Norwalk, CT) according to the following formula:

$$\text{lycopene content (\%)} = \frac{\text{(absorbance at 472 nm diluted-fold)}}{3450 \times \text{specimen weight (g)}} \times 100\% \quad [1]$$

Figure 1 shows the UV-visible absorption spectrum of a hexane solution of LC. The absorption spectrum coincided almost exactly with the three maximal characteristic peaks for *trans*-lycopene (λ = 446, 472, 505 nm). The measurement conditions were: scan speed 90 nm/min and data interval of 1 nm. β-Carotene was purchased from Sigma Chemical Co. (St. Louis, MO). Pyrogallol was purchased from Beijing Chemical Co. Ltd. (Beijing, China). Luminol and 1,10-phenanthroline were from Sigma Chemical Co. 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was from Wako Chemical Co. (Tokyo, Japan). Evening primrose oil (γ-linolenic acid content >20%), NaOCl, H_2O_2, HCl, and CuCl were bought from Shanghai Fuzhou Lu Chemicals Co. (Shanghai, China). NaOCl and H_2O_2 were required to be fresh. Carbonic acid-buffered saline solution (pH = 10.2) (CBSS) and triply-distilled water were prepared in our laboratory. All the chemicals were analytical grade.

Antioxidant properties were assessed by a SH-G Biochemistry Chemiluminescence Meter (BCM) of Shanghai No. 6 In-

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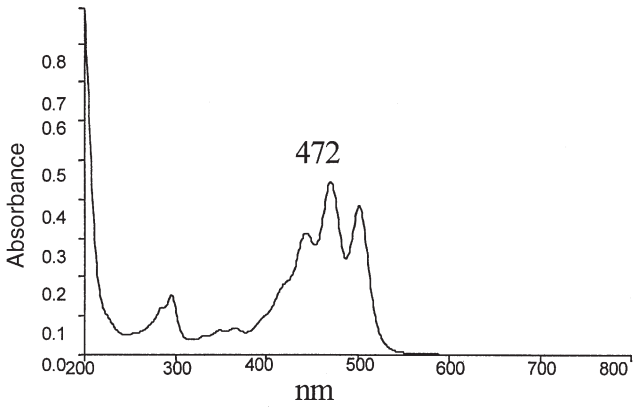

FIG. 1. Ultraviolet/visible spectrum of lycopene concentrate.

strument Factory. The BCM is composed of three main parts: the automatically rotating sample support, in which 12 sample cells (glass tube, diameter = 10 mm, height = 20 mm) can be put, the chemiluminescene monitor, and the accompanying data processor. Each sample cell can automatically rotate with the sample support rotation at a set time interval. Thus, every sample cell can cross the monitor at a set time interval. So, when testing, the chemiluminescence value (CL) could be recorded in the accompanying data processor and be displayed on the processor screen at a set time interval. Four model systems tested here were as follows.

Superoxide anion assay. Superoxide anions were generated by pyrogallol autoxidation (8) The reaction mixture contained 50 μL pyrogallol (1 mmol/L), 700 μL CBSS (pH = 10.2), and 20 μL luminol (1 mmol/L). A sample cell loaded with the mixture was first placed into the BCM. When the cell crossed the monitor, an indicated concentration of sample was injected into the cell *in situ*. The CL was simultaneously recorded in the processor and then was recorded once every 6 s (CBSS instead of specimen was present in control). The scavenging rate was obtained according to the formula:

$$\text{scavenging rate}\ (\%) = \frac{(\text{CL}_{\text{control}} - \text{CL}_{\text{sample}})}{\text{CL}_{\text{control}}} \times 100\% \qquad [2]$$

Hydroxyl radical assay. Hydroxyl radicals were generated by a Fenton-type reaction. (9). The reaction mixture included 20 μL $FeCl_2$ (1 mmol/L), 30 μL 1,10-phenanthroline (1 mmol/L), 800 μL CBSS, and 50 μL H_2O_2 (0.6%). A sample cell loaded with the mixture was first placed into the BCM. When the cell crossed the monitor, an indicated concentration of sample was injected into the cell *in situ*; CBSS instead of sample was present in control. The CL was simultaneously recorded in the processor and then was recorded once every 6 s. The scavenging rate was obtained according to Equation 2.

Assay of singlet oxygen in the system of OH^--NaOCl-H_2O_2 (10). Singlet oxygen was generated chemically by the reaction between NaOCl and H_2O_2 in a pH = 8 solution at room temperature. For testing purposes, sample cells loaded with 200 μL NaOCl (1.4 mmol/L), 20 μL NaOH (5 mmol/L), and a series of specimens containing selected concentrations of LC were first put into BCM. When a sample cell crossed the monitor, the 600 μL H_2O_2 (0.6%) was injected *in situ*. At the same time, a chemiluminescence signal of the reaction systems was produced and was recorded in the processor every 3 s (CBSS instead of sample was present in control). The scavenging rate was obtained according to the Equation 2.

Assay of AAPH-initiated lipid peroxidation in γ-linolenic acid. Lipid peroxidation was generated from the oxidation of γ-linolenic acid induced by AAPH. The reaction mixture contained 100 μL evening primrose oil (γ-linolenic acid content >20%), 20 μL 1 mmol/L luminol, and 100 μL AAPH (5 mg/mL) in a sample cell. The mixture was incubated in a preheated oven (Shanghai No. 3 Chemical Equipment Factory, (Shanghai, China) at 37°C for 0.5 h. After that, the test began. A cell loaded with the mixture was first placed into the BCM. When the cell crossed the monitor, an indicated concentration of sample was injected into the cell *in situ* (CBSS instead of sample was present in control). The CL was simultaneously recorded in the processor and then was recorded every 6 s (CBSS instead of sample was present in control). The temperature of assay was 38°C. The inhibiting rate was obtained according to Equation 2.

High-performance liquid chromatography (HPLC). Tomato paste contains a number of other antioxidant carotenoids besides lycopene, like β-carotene, phytoene, phytofluene, ζ-carotene, γ-carotene, and neurosparene. So, LC may contain several carotenoids. To identify its composition, we analyzed the LC using high-performance liquid chromatography (HPLC) (Waters Co., Milford, MA) on a 5-μm particle size Vydac C18 column (Hesperia, Palo Alto, CA) with the solvent mixture of diisopropylethylamine and hexane (1:100, vol/vol) and monitored peak signals at 472 nm.

RESULTS AND DICUSSION

Determination of free radical models. The free radical models of superoxide anion, hydroxyl radical, lipid peroxidation, and singlet oxygen are illustrated in Figure 2, which reflects the changes in concentration of free radicals with time. Superoxide anion, hydroxyl radical, and lipid peroxidation remained relatively stable (Figs. 2A–C), but singlet oxygen was unstable (Fig. 2D). From Figure 2D, one can see that H_2O_2 or NaOCl alone could not produce a chemiluminescent signal. Only when NaOCl and H_2O_2 were mixed together did the signal of singlet oxygen appear. CL increased and decayed rapidly in the first 6 s. After 6 s, it became relatively stable. In the following testing, the CL of various specimens at 6 s was recorded, based on which the scavenging effect of the LC toward singlet oxygen was evaluated.

Antioxidant properties of LC. (i) Superoxide anion. Superoxide anions are important oxygen radicals that can occur during *in vivo* metabolism. But if *in vivo* superoxide anions are present in a higher than allowable concentration, they will destroy macromolecules like protein, DNA, and the like (11). Thus, it is beneficial to explore the effect of LC on superox-

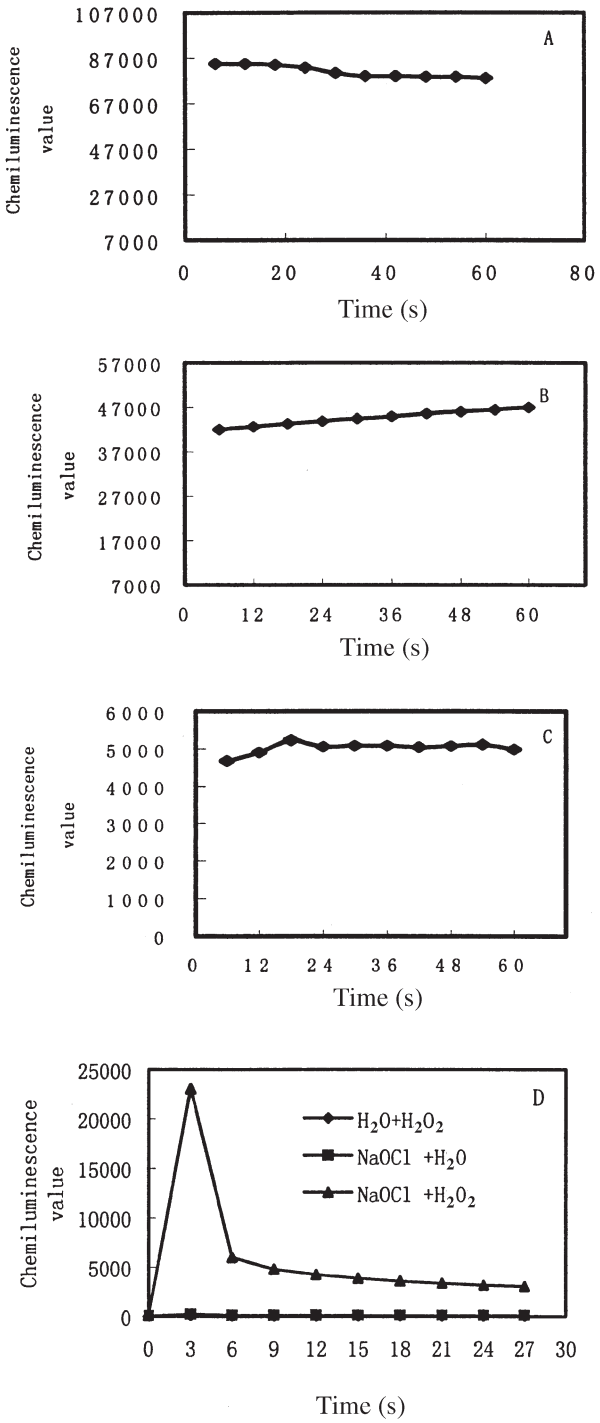

FIG. 2. Four free radical models. (A) Superoxide anion, (B) hydroxyl radical, (C) lipid peroxidation, (D) singlet oxygen.

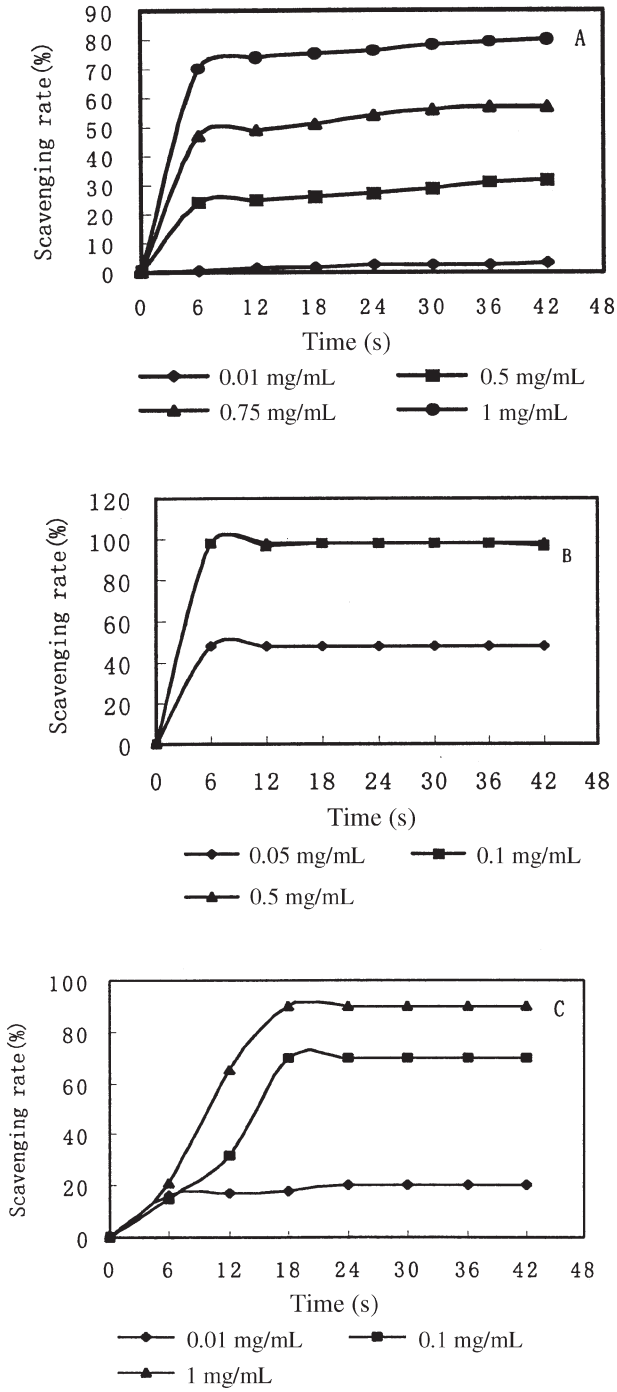

FIG. 3. Plots of radical scavenging and lipid peroxidation inhibition by lycopene concentrate. (A) Superoxide anions, (B) hydroxyl radicals, (C) lipid peroxidation.

ide anions. In this testing, although superoxide anions generated from pyrogallol autoxidation gave out a weak light, it could be enhanced by luminol, and thus a strong chemiluminescence signal could be seen on the processor screen. After the addition of LC, the signal intensity decreased sharply. The reduced signal intensity represented the scavenging capability of specimens (Fig. 3A). Scavenging curves reached a constant value in just 6 s after addition of the LC and then rose slightly with time (Fig. 3A). The relation between scavenging rate and concentration of LC is shown in Figure 4A. Scavenging rate rose linearly with the concentration of LC and reached 60–80% when the concentration was more than 0.75

mg/mL. According to the calculation of efficient concentration (EC_{50}), defined as the concentration of antioxidant necessary to inhibit 50% of the CL, which represented the free radical scavenging activity of antioxidant, EC_{50} of LC toward superoxide anions was 0.75 mg/mL.

(ii) Hydroxyl radical. Hydroxyl radicals are the most aggressive oxygen species in *vivo* (12), and they are the most important killer for biomolecules. We designed a direct reaction between LC and hydroxyl radicals. The curves of hydroxyl radical by LC were expressed in Figure 3B. Six seconds was enough for effective scavenging of hydroxyl radicals (Fig. 3B). The effect of various concentrations of LC on hydroxyl radicals is shown in Figure 4B. From Figure 4B, the EC_{50} of LC toward hydroxyl radicals was 0.05 mg/mL and when the concentration of LC was increased, scavenging rate was up to almost 100%. So, LC was a very effective hydroxyl radical scavenger. Rao and Agarwal (1) observed that dietary supplementation of lycopene from traditional tomato products including tomato juice and spaghetti sauce increased lycopene concentrations in their subjects' plasma and reduced oxidative damage to lipids, proteins, and DNA. The importance of lycopene may be mainly attributed to its effective antioxidant capability against OH^-.

(iii) Lipid peroxidation. Lipid peroxidation (13) is a common product of oxidative stress in biological tissues such as lipoproteins, liposomes, microsomes, and membranes. The ability to reduce lipid peroxidation (13) has become an important factor in examining the biological benefits of antioxidants. In our laboratory, lipid peroxidation was generated from the oxidation of γ-linolenic acid induced by AAPH. γ-Linolenic acid is an unsaturated acid that can be found in animal cell membranes. The curves for inhibition of the lipid peroxidation reaction by LC are shown in Figure 3C. The time required for effectively inhibiting lipid peroxidation was 18 s. The effect of various concentrations of LC on lipid peroxidation is displayed in Figure 4C. The EC_{50} of LC toward lipid peroxidation of γ-linolenic acid was 0.1 mg/mL.

(iv) Singlet oxygen. Singlet oxygen (^1O) (14), although not technically a free radical, is a very reactive high-energy and short-lived oxygen species that can react with biomolecules. Figure 4D shows the scavenging capability of LC toward singlet oxygen. LC is effective at scavenging singlet oxygen at 1 mg/mL. Singlet oxygens have been studied in the system of thermodissociation of the endoperoxide of 3,3′-(1,4-naphthylidene)dipropionate. (6). In our laboratory, singlet oxygens were generated chemically by the reaction between NaOCl and H_2O_2 in a pH = 8 solution and could be effectively scavenged by LC. Our result was consistent with the work of Di Mascio *et al*. (6).

From our experimental results, one can conclude that LC is effective in scavenging such ROS as superoxide anion, hydroxyl radical, singlet oxygen, and lipid free radical. These results favorably supported the significant role of lycopene-rich foods in prevention of chronic diseases and cancers, which has been observed in cell culture, animal experiments, and clinics (15).

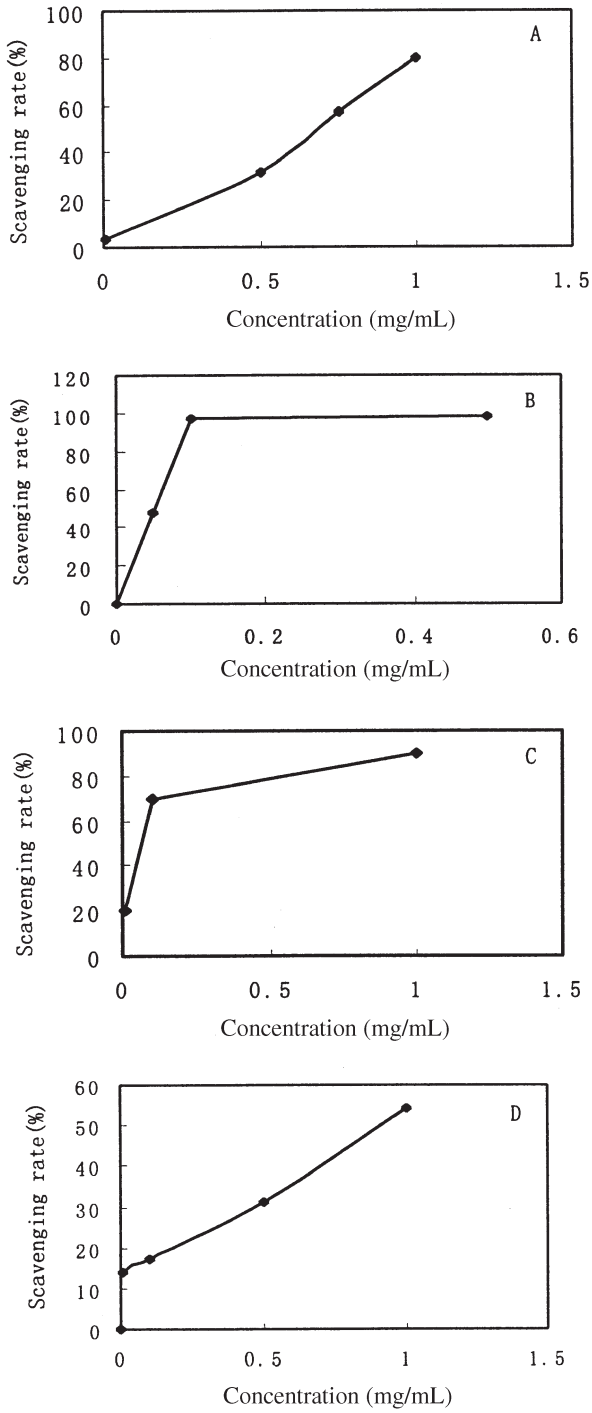

FIG. 4. Effect of various concentrations of lycopene concentrate on scavenging free radicals. (A) Superoxide anions, (B) hydroxyl radicals, (C) lipid peroxidation, (D) singlet oxygens.

HPLC. Figure 5 is a high-pressure liquid chromatogram of LC (part A) and standard β-carotene (part B). In Figure 5A, there are two peaks: the main peak at 5.9 min is ascribed to lycopene, and the small peak at 4.2 min is ascribed to β-carotene according the HPLC of its standard specimen. Lycopene was about 99% of all the carotenoids in LC and β-carotene was about 1%. Other carotenoids may be removed

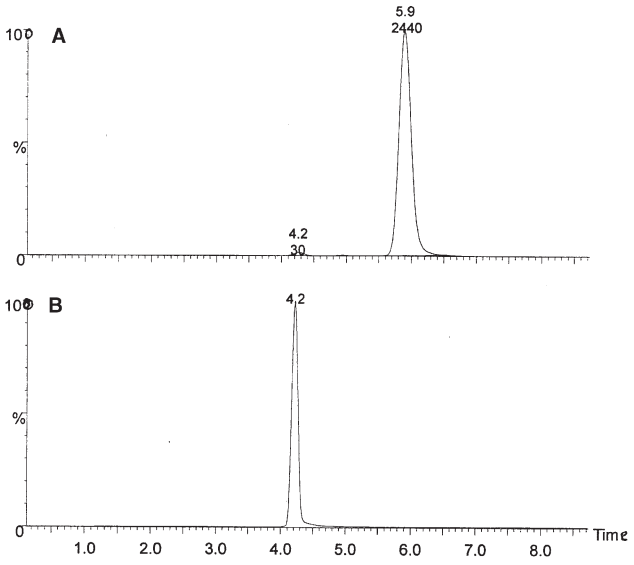

FIG. 5. High-performance liquid chromatograms of lycopene concentrate (A) and standard β-carotene (B).

during pretreatment. These results basically were consistent with the work of Bushway (16). Thus, the main antioxidant capability of LC may be ascribed to lycopene. Of course, the small quantity of β-carotene may contribute antioxidant capability to some extent. Besides, in LC, there were nearly 50% other lipid-soluble substances, which may consist of oil resins, waxy hydrocarbons, phospholipids, etc. Studies on identifying these complex components and on purifying lycopene to more than 90% are in progress in our laboratory and so are the synergistic effects of lycopene and other carotenoids.

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