Evaluation of Radical Scavenging Activity of Fresh and Air-Dried Tomatoes by Three Model Reactions

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The radical scavenging activity and the antioxidant content of fresh and air-dried tomatoes were investigated. Tomato halves were dried in a pilot-scale dryer under the following conditions: air temperature, 80 °C; air flow rate, 1.5 m/s; drying time, 400 min; final moisture, 25%. Carotenoid (lycopene, β-carotene, lutein) and ascorbic acid were analyzed by HPLC with a spectrophotometric and an electrochemical detector, respectively. Total phenolics were determined by using the Folin−Ciocalteu reagent. The radical scavenging activity was studied in three model systems: (a) the xanthine oxidase and xanthine system, which generates superoxide radical and hydrogen peroxide; (b) the 3-morpholinosydnonimine system, which releases spontaneously superoxide radical and nitrogen monoxide, forming peroxynitrite; (c) the linoleic acid and $CuSO_4$ system, which promotes lipid peroxidation. These model systems allow the simulation of key reactions involved in the pathogenesis of certain chronic diseases and may be related to the in vivo activity of tomato antioxidants. Hence, these measurements can be used for optimizing tomato processing and storage. The drying process resulted in a decrease of ascorbic acid content, whereas phenol reagent reducing compounds increased. Carotenoid levels were substantially unchanged upon drying. Fresh and air-dried tomato extracts could act as radical scavengers both in the reactive oxygen species-mediated reactions and in lipid peroxidation. Drying affected the antioxidant effectiveness as measured in the xanthine/xanthine oxidase system, which was found to be the most sensitive method for the measurement of tomato antioxidant activity (lower I_{50}) but retained the antioxidant effectiveness in the other two systems.

Keywords: *Tomato (Lycopersicon esculentum); air-drying; radical scavenging activity; xanthine oxidase; peroxynitrite; lipid peroxidation*

INTRODUCTION

A number of factors are known to promote free radical-mediated reactions in food systems during processing and storage, causing oxidative degradation and in some cases the formation of toxic end products. In fruits and vegetables the loss of natural antioxidants is of great nutritional importance because these compounds are believed to inhibit in vivo the initiation and progression of a range of human diseases, such as coronary heart disease, carcinogenesis, neuronal disease, cataracts, and age-related macular degeneration (Elstner, 1990; Diplock et al., 1998).

Studies dealing specifically with the relevance of tomato carotenoids, and in particular lycopene, to human health have been reviewed by Stahl and Sies (1996) and Gerster (1997). Remarkable inverse relationships between tomato intake or lycopene serum level and risk have been observed for digestive tract, bladder, pancreatic, prostate, and cervical cancer and cardiovascular disease. These properties have been related to carotenoid antioxidant activity and influence on cell growth and intercellular communication. Few studies have dealt with the tomato content of noncarotenoid antioxidants and their relationships with in vivo properties. The ascorbic acid content of tomato has been shown to provide a significant contribution to dietary intake (Davies and Hobson, 1981). The qualitative and quantitative distribution of phenolic compounds has been recently investigated (Herrmann, 1976; Fleuriet and Macheix, 1985; Hertog et al., 1992; Crozier et al., 1997; Vinson et al., 1998).

The effects of processing on tomato antioxidant components have been studied by examining the changes in the levels of carotenoids, ascorbic acid, and quercetin. Thermally processed tomato-based commercial products (juice and concentrates) were found to have a high carotenoid content with respect to raw tomato. However, the effect of processing on carotenoid level could not be quantified because the raw and processed tomatoes analyzed were not the same genetic variety (Tonucci et al., 1995). Stewing process (8 min) was found to retain the levels of individual carotenoids in tomato (Khachik et al., 1992). Pasteurization (121 °C, 42 s) and canning of tomato were shown to cause no damage to β-carotene (Dietz and Gould, 1986). Tomato juice manufacture was shown to achieve an average ascorbic acid retention of 63−70%. Higher losses occurred during the manufacture of tomato concentrates with respect to tomato juice or whole canned tomatoes (Gould, 1992). Cooking procedures such as boiling (15 min), microwaving (at 800 W, for 1.3 min, in water), and frying (for 2.5−3 min in sunflower oil) resulted in a lowered conjugated quercetin

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content in tomatoes. This effect was attributed to flavonoid breakdown and/or to the extraction of conjugated quercetin by hot water and sunflower oil (Crozier et al., 1997).

A thorough knowledge of the total antioxidant activity of tomato is still to be developed. Furthermore, information on the fate of tomato antioxidant activity during processing is lacking. The aim of the present work was to evaluate the antioxidant content and the total antioxidant activity of raw and air-dried tomato products in three model systems simulating different types of free radical-mediated reactions, and to develop analytical indices for tomato technology optimization.

MATERIALS AND METHODS

Materials. Tomato fruits (*Lycopersicon esculentum*) of Rita variety were purchased from a local market. Linoleic acid, xanthine, α-keto-γ-methiolbutyric acid (KMB), and the reference samples for lycopene (90−95% purity), β-carotene (95% purity), lutein (70% purity), ascorbic acid (>99.0% purity), and chlorogenic acid (>95% purity) were obtained from Sigma and used without further purification. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, >97% purity) was obtained from Aldrich. An aqueous solution (10% w/v) of especially purified Tween 20 (for membrane research) and xanthine oxidase from cow milk (XOD) were obtained from Boehringer Mannheim. 3-Morpholinosydnonimine hydrochloride, $C_6H_{10}N_4O_2{\cdot}HCl$ (sin-1), was a gift from Dr. R. Grewe, Fa. Hoechst AG, Frankfurt. Folin−Ciocalteu reagent and HPLC grade unstabilized tetrahydrofuran (THF) were purchased from BDH. HPLC grade methanol and petroleum ether (PE) were supplied by Merck.

Sampling Procedure. Fruits were selected on the basis of size and color (as a ripening degree index). Color was measured by a tristimulus Cr-210 Minolta Chromameter calibrated with a white standard and expressed as the ratio of a^* to b^* Hunter values, which is the ratio of red to green components. On each individual fruit, color measurements were performed at three different positions of the skin and the average a^*/b^* was obtained. Homogeneous lots of 20 fruits with 6.5 cm of diameter and a^*/b^* ranging from 1.6 to 2.0 were grouped.

Drying. For each drying trial two homogeneous lots were used. Fruits were cut into halves and separated from parenchyma and seeds. The first lot of tomato halves was dried in a pilot plant (Figure 1) under the following conditions: air temperature, 80 °C, with an absolute humidity of ∼0.01% kg of water/kg of dry air; air flow rate, 1.5 m/s; final sample moisture, 25%; drying time, 400 min. During drying, weight loss and temperature were monitored. Air-dried tomato halves were homogenized by a commercial blender (Waring 32B/79) at a moderate speed for 1 min. The second lot of tomato halves was homogenized by an Omni-mixer (17106 Sorvall DuPont Instrument) for 2 min and used as a reference for fresh tomato. Fresh and air-dried tomato homogenates were frozen in liquid nitrogen and stored under vacuum in the dark at −20 °C. Prior to extraction and analysis, air-dried tomato homogenate (0.3 g, dry weight) was added to 5 mL of distilled water and rehydrated by keeping the mixture at 4 °C for 30 min under nitrogen.

Total Solids. The solids content was gravimetrically determined by drying a 5.0 g aliquot in a vacuum oven at 70 °C to constant weight (12−14 h).

HPLC Equipment. The HPLC equipment consisted of an L-7100 Merck Hitachi pump, an L-7400 Merck Hitachi UV−vis detector or an EG&G instruments (Princeton Applied Research) Model 400 electrochemical detector, and a D-7500 Merck Hitachi integrator.

GC Equipment. The GC equipment consisted of a Varian Aerograph 3300 with a Varian integrator; column, $^1/_8$ in. × 100 cm aluminum oxide deactivated; column temperature, 60 °C; injection temperature, 80 °C; FID temperature, 225 °C.

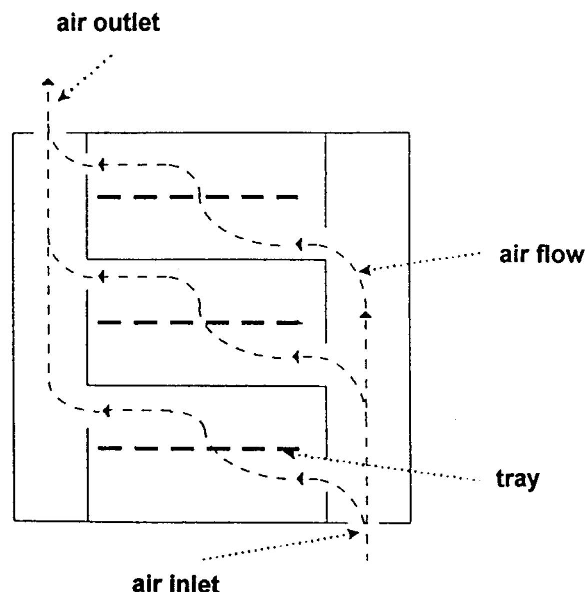

Figure 1. Schematic representation of the pilot plan used for tomato drying. Air temperature and air flow rate were 80 °C and 1.5 m/s, respectively.

UV−Vis Spectrophotometer. UV−vis measurements were performed with a Jasco UVDEC-610 spectrophotometer.

Carotenoids. For carotenoid extraction tomato homogenate (0.3 g, dry weight) was added to 15 mL of THF. The mixture was kept refrigerated in an ice bath and mixed by an Ultra-Turrax homogenizer (T25 Janke & Kunkel IKA Labortechnik) under nitrogen at moderate speed for 15 s. The extract was centrifuged (at 12000*g* at 5 °C for 10 min), and residual solids were re-extracted with 15 mL of THF. Combined THF extracts and residual solids were centrifuged (at 12000*g* at 5 °C for 10 min). Supernatant was quantitatively recovered and partitioned into 60 mL of PE and 30 mL of 20% NaCl in water. The organic layer was quantitatively transferred into a volumetric flask and brought up to 100 mL with PE. Aliquots of 10 mL each were removed, dried in separate tubes by nitrogen, and stored under nitrogen, in the dark at −20 °C. Extractions were carried out in duplicate. Carotenoids were determined by HPLC according to the method of Riso and Porrini (1997). A Vydac 201TP54 C_{18} column (250 × 4.6 mm), equipped with a C_{18} precolumn, was used. Chromatographic separation was performed with methanol/THF (stabilized by 0.1% butylated hydroxytoluene) 95:5 as an eluent, under isocratic conditions, 1.0 mL/min flow rate, at room temperature. Dried aliquots of tomato extract were redissolved in 1 mL of stabilized THF and 4 mL of eluent. Lycopene, β-carotene, and lutein were identified and quantified by calibration curves obtained with pure compounds using a UV−vis detector set at 454 nm.

Ascorbic Acid. For ascorbic acid extraction tomato homogenate (0.6 g, dry weight) was added to 50 mL of distilled water. The mixture was kept refrigerated in an ice bath and mixed by an Ultra-Turrax homogenizer (T25 Janke & Kunkel IKA Labortechnik) under nitrogen at moderate speed for 30 s. The mixture was filtered (0.45 μm) and immediately analyzed. Extractions were carried out in duplicate. Ascorbic acid was determined by HPLC according to the method of Mannino and Pagliarini (1988). A Bio-Rad Fruit Quality Analysis column (100 × 7.8 mm) was used. Chromatographic separation was performed with 1 mM H_2SO_4 as an eluent, under isocratic conditions, 0.8 mL/min flow rate, at room temperature. Ascorbic acid was detected by an electrochemical detector operating at a potential of 800 mV vs Ag/AgCl.

Total Phenolics. For total phenolics extraction, tomato homogenate (0.3 g, dry weight) was added to either 10 mL of 0.1 M potassium phosphate buffer, pH 7.4, or 10 mL of 0.5% tartaric acid, 0.2% $Na_2S_2O_5$, 12% ethanol, and 2% 1 N NaOH, pH 3.2. The mixture was kept refrigerated in an ice bath, mixed by an Ultra-Turrax homogenizer (T25 Janke & Kunkel

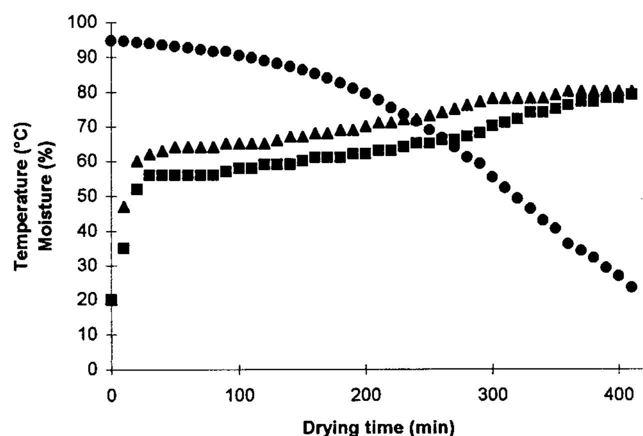

Figure 2. Kinetics of water loss (●), pulp temperature (■), and surface temperature (▲) during air-drying.

IKA Labortechnik) under nitrogen, at moderate speed, for 1 min, and filtered through a Whatman No. 4 filter paper. Total phenolics were determined after a purification step as reported by Di Stefano and Cravero (1991). The filtrate was diluted with 1 N H_2SO_4 (1:1), and 2 mL was loaded on a C_{18} Sep-Pak cartridge (WAT 051910 Waters) activated with 2 mL of methanol and 5 mL of distilled water. The cartridge was washed by 2 mL of 0.1 N H_2SO_4 prior to phenolics elution with 2 mL of methanol and 5 mL of distilled water. Combined methanol and water fractions were analyzed for total phenolic compounds content by visible spectrophotometry at 760 nm, after reaction with Folin−Ciocalteu reagent. Extractions were carried out in quadruplicate. Results were calculated by a calibration curve obtained with chlorogenic acid.

Antioxidant Activity. The antioxidant activity was evaluated in three model systems. For systems 1 and 2 fresh and rehydrated tomato samples were kept refrigerated in an ice bath, mixed by an Ultra-Turrax homogenizer (T25 Janke & Kunkel IKA Labortechnik) under nitrogen, at moderate speed, for 1 min, and analyzed after dilution with distilled water. For system 3 samples were extracted with THF and PE as for carotenoid extraction. The first system contained 0.1 M phosphate buffer, pH 7.4; 0.5 mM xanthine (in 10 mM NaOH); 0.08 U XOD; 1.25 mM KMB; and various concentrations of tomato water homogenate. The reaction was carried out for 30 min at 37 °C and followed either by gas chromatography [according to the method of Kruedener et al. (1995)] or by an oxygen electrode (Rank Brothers) to measure ethene release from KMB and oxygen consumption, respectively. The second system contained 0.1 M phosphate buffer, pH 7.4; 0.01 mM sin-1; 1.25 mM KMB; and various concentrations of tomato water homogenate. The reaction was carried out for 30 min at 37 °C and followed by measuring ethene release from KMB. The third system contained 0.05 M phosphate buffer, pH 7.0; 1% Tween 20; 1.2 mM linoleic acid; 5 μM $CuSO_4$; and various concentrations of tomato THF extracts. The reaction was carried out for 60 min at 37 °C and followed by UV−vis spectrophotometry to measure the formation of conjugated dienes of hydroperoxides and lycopene degradation.

Data Expression. All results were obtained from a minimum of four independent experiments and averaged. Data were expressed on a dry weight basis.

RESULTS

Moisture removal from tomato and temperature profile during drying were monitored at 10 min intervals (Figure 2). The process was stopped at ~25% moisture content. According to our previous data on tomato water adsorption equilibrium, a moisture content of 25% corresponds to a water activity value of 0.75, at 25 °C (Zanoni et al., 1999). Hence, the final air-dried product can be considered as a shelf-stable product at room temperature.

Table 1. Antioxidant Content in Tomato prior to and after Air-Drying

	antioxidant content[a] (mg/kg, dry wt)	
	fresh	air-dried
carotenoid		
lycopene	850 ± 50	830 ± 50
β-carotene	74 ± 10	83 ± 3
lutein	7.7 ± 2	16 ± 1
phenol reagent reducing compounds		
extracted with buffer solution at pH 3.2, 12% ethanol	2700 ± 500	3700 ± 800
extracted with buffer solution at pH 7.4	3600 ± 300	5600 ± 100
ascorbic acid	3300 ± 100	400 ± 60

[a] Data expressed as average ± standard deviation.

The antioxidant content of tomato was analyzed prior to and after air-drying (Table 1). Carotenoid levels were substantially unaffected by air-drying, except for an increase in lutein content, probably due to an increased extractability in air-dried samples. Conversely, the ascorbic acid content decreased in air-dried samples. These results are in agreement with previous indications on carotenoid and ascorbic acid thermal stability (Dietz and Gould, 1986; Gould, 1992; Khachik et al., 1992; Tonucci et al., 1995). The phenol reagent reducing compounds extracted either with 12% ethanol in buffer solution at pH 3.2 or with buffer solution at pH 7.4 were higher in the air-dried tomato samples. This result might be due to the release of cell wall phenolics or an increase in the number of free hydroxyl phenol groups. In fact, during drying flavonoid glycosides could have been hydrolyzed to their aglycons, releasing free phenol hydroxyl groups [flavonoid glycosides are hydrolyzed in 1.2 M HCl/50% methanol, at 90 °C for 2 h, as described in Hertog et al. (1992)]. Fresh tomatoes were found to contain as much as 9.5 μmol/g (dry weight) of free phenols and 18.9 μmol/g (dry weight) of free plus conjugated phenols, as measured after acid hydrolysis (Vinson et al., 1998). Interestingly, our values obtained by extracting with phosphate buffer at pH 7.4 were 10.2 μmol/g (dry weight) for fresh tomato and 15.8 μmol/g (dry weight) for air-dried tomato, approaching the values for free phenols and free plus conjugated phenols, respectively.

The effect of air-drying on tomato antioxidant activity was studied by using three model systems to differentiate the free radical chain reaction patterns, which depend on the chain initiator and propagators.

The xanthine/XOD system produces superoxide radical and hydrogen peroxide, releasing ethene from KMB (Meyer et al., 1995). Fresh and air-dried tomato water homogenates were found to inhibit ethene release in a concentration-dependent manner. The effectiveness of inhibition of fresh tomatoes was higher than that of air-dried tomatoes at low concentrations, whereas at the highest concentration a saturation effect was observed and differences disappeared (Figure 3). The I_{50} values of fresh and air-dried tomatoes were 625 and 1200 μg (dry weight), respectively. The antioxidant activity was compared to that of the water-soluble α-tocopherol analogue Trolox, which showed an I_{50} value of 150 nmol. In terms of Trolox equivalents tomato antioxidant activity was 240 nmol of Trolox/mg for fresh tomato (dry weight) and 125 nmol of Trolox for air-dried tomato (dry weight). To verify whether the inhibition of ethene release was due to the scavenging of superoxide radical

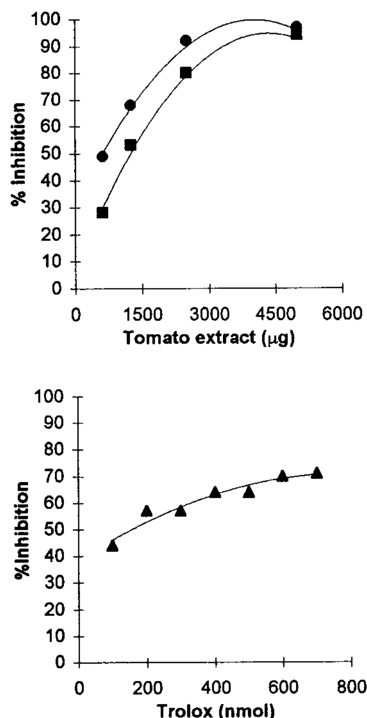

Figure 3. Inhibitory effect of fresh (●) and air-dried (■) tomato samples and of the standard Trolox (▲) on KMB fragmentation by XOD/xanthine system (as percent of the control reaction rate).

Table 2. Oxygen Uptake in the XOD/Xanthine System in the Presence and Absence of Fresh and Air-Dried Tomato Samples

sample	oxygen uptake[a] (nmol/min)
control	43.0 ± 0.9
fresh tomato	40.1 ± 1
air-dried tomato	41.6 ± 3.5

[a] Data expressed as average ± standard deviation.

and/or hydrogen peroxide or to a direct inhibition of the XOD catalyst, the reaction was followed potentiometrically with an oxygen electrode. Oxygen uptake was not influenced by tomato homogenates at the concentration that caused a 68% decrease of ethene release, suggesting that inhibition is the result of the reactive oxygen species scavenging effect (Table 2).

The sin-1 system generates superoxide radical and nitrogen monoxide, forming peroxynitrite, which releases ethene from KMB (Hippeli et al., 1997). Fresh and air-dried tomato water homogenates were found to inhibit ethene release to the same degree with a dose-dependent effect. The I_{50} values of tomato samples corresponded to 4000 μg (dry weight) and that of Trolox was 470 nmol. These values were higher than those found in the xanthine/XOD/KMB system (Figure 4). In terms of Trolox equivalents tomato antioxidant activity was 120 nmol of Trolox/mg (dry weight).

The linoleic acid/copper system promotes lipid peroxidation. Fresh and air-dried tomato lipophilic extracts were found to have the same dose-dependent inhibitory effect with an I_{50} value of 1900 μg (dry weight) (Figure 5). The I_{50} value of Trolox was 7.3 nmol, which is very low in comparison with that found in the previous model systems. Hence, tomato antioxidant activity was 3.8 nmol of Trolox/mg (dry weight). Lycopene degradation, evidenced by the modification of visible spectra in the region 400−600 nm, occurred in parallel with the

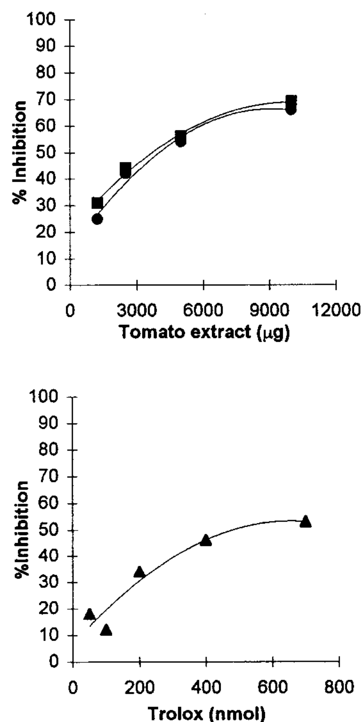

Figure 4. Inhibitory effect of fresh (●) and air-dried (■) tomato samples and of the standard Trolox (▲) on KMB fragmentation by sin-1 (as percent of the control reaction rate).

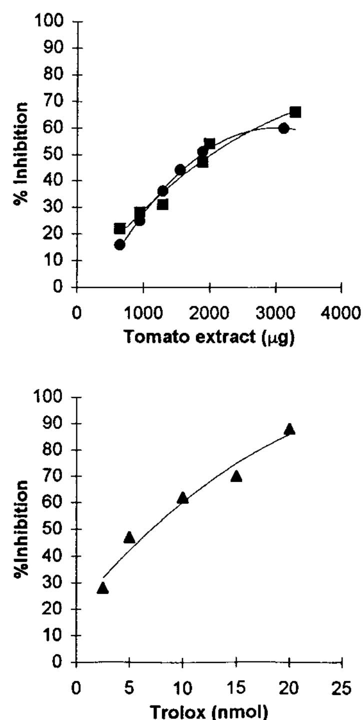

Figure 5. Inhibitory effect of fresh (●) and air-dried (■) tomato samples and of the standard Trolox (▲) on conjugated dienes of hydroperoxides formation in linoleic acid/$CuSO_4$ system (as percent of the control reaction rate).

antioxidant effect (Figure 6). The decay of the absorbance at 510 nm, which was monitored as an index of lycopene degradation, was processed statistically. An exponential curve represented the best fit for the experimental values, indicating that lycopene degradation followed first-order kinetics. Raw and air-dried tomato samples showed substantially the same lycopene degradation constant rate.

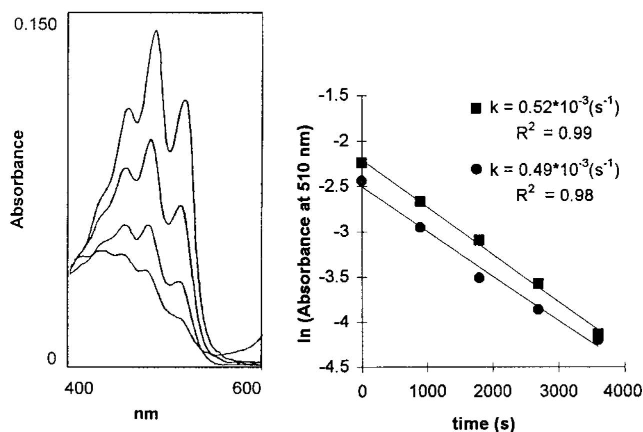

Figure 6. (Left) Modifications of visible spectra during the copper-catalyzed linoleic acid oxidation in the presence of raw tomato extract. Spectra were recorded at 0, 15, 30, and 60 min from the addition of copper (from top to bottom). The air-dried tomato extract showed spectral changes similar to those of raw tomato extract. (Right) Lycopene degradation kinetics during the copper-catalyzed linoleic acid oxidation in the presence of raw tomato extract (●) or air-dried tomato extract (■). Rate constants (*k*) were calculated by fitting data by first-order kinetics.

DISCUSSION

The present work evidenced that tomato extracts can act as radical scavengers both in the reactive oxygen species-mediated reactions and in lipid peroxidation. The radical scavenging activity of tomato water and acetone extracts has previously been observed by using a synthetic hydrophilic peroxyl radical generator, 2,2′-azobis(2-amidinopropane) dihydrochloride, as a catalyst and β-phycoerythrin as a target (Wang et al., 1996). On the contrary, intriguing results have been reported on the effect of tomato methanolic/water (1:1) fraction on microsome peroxidation: an inhibitory effect has been observed on the peroxidation of cytochrome P450s isoenzyme-enriched microsomes, but no effect has been observed on the peroxidation of microsomes with negligible cytochrome P450s content (Plumb et al., 1997). In a recent study tomato methanolic/water (1:1) fraction was found to inhibit the copper-catalyzed oxidation of isolated lower density lipoprotein (LDL plus VLDL) (Vinson et al., 1998). Another biological model based on 7,12-dimethylbenz[*a*]anthracene-induced rat mammary tumors has been developed for studying the role of lycopene-enriched tomato oleoresin in the inhibition of cancer initiation and progression. The anticancer effect has been evidenced, although the protective mechanism has not been understood (Sharoni et al., 1997).

The model systems used in the present study also allow the simulation of key reactions involved in the pathogenesis of certain chronic disease and may be related to the in vivo activity of tomato antioxidants (Elstner, 1990). XOD is present in several types of cells and is thought to be a relevant source of reactive oxygen species in vivo after reperfusion of ischemic tissues (Hippeli and Elstner, 1997). Peroxynitrite, which was produced in vitro by sin-1 decomposition, can be generated in vivo under pathological conditions by a reaction between superoxide radical and nitrogen monoxide, which are enzymatically produced, and has been associated with damage to endothelial cells and erythrocytes and with neurodegenerative processes (Hippeli and Elstner, 1997). Lipid peroxidation is involved in oxidative processes in vivo, causing damage to LDL, which promotes the development of atherosclerosis, and to membranes (Halliwell et al., 1995).

Air-drying processes are associated with the risk of oxidative and thermal damage. The present study evidenced that most of the potential antioxidant properties of tomato are still present in air-dried product, except for the ascorbic acid content and the antioxidant effectiveness in the xanthine/XOD system, which was found to be the most sensible method for the measurement of tomato antioxidant activity within our model systems (in fact, the I_{50} value of fresh tomato was about 3- or 6-fold lower than those observed in the other systems). These latter indices could therefore be used for monitoring the effect of processing and storage on tomato properties. However, further biological models should be used to study the healthy properties of tomato antioxidants, because the beneficial effect is likely to be not exclusively related to a single component or to the antioxidant effectiveness in a single model system. In this view, further investigations are needed to establish the effect of processing on tomato antioxidant properties and to define analytical indices for tomato technology optimization.

ABBREVIATIONS USED

KMB, α-keto-γ-methiolbutyric acid; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; XOD, xanthine oxidase; sin-1, 3-morpholinosydnonimine hydrochloride; THF, tetrahydrofuran; PE, petroleum ether.

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