Antioxidant Activity of Tomato Products As Studied by Model Reactions Using Xanthine Oxidase, Myeloperoxidase, and Copper-Induced Lipid Peroxidation

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The antioxidant content and activity of commercial tomato products differing in variety and processing were studied. Two procedures for extracting hydrophilic and lipophilic antioxidants, namely, two-step 0.1 M phosphate buffer (pH 3.0 and 7.4) extraction and tetrahydrofuran extraction followed by petroleum ether fractionation, were developed. Carotenoids (lycopene, β-carotene, and lutein) and ascorbic acid were analyzed by HPLC with spectrophotometric and electrochemical detectors, respectively. Total phenolics were determined by using the Folin−Ciocalteu reagent. The antioxidant activity was studied by the following three model systems: (a) the xanthine oxidase (XOD)/xanthine system, which generates superoxide radical and hydrogen peroxide; (b) the myeloperoxidase (MPO)/NaCl/H_2O_2 system, which produces hypochloric acid; and (c) the linoleic acid/$CuSO_4$ system, which promotes lipid peroxidation. Results showed that the hydrophilic and lipophilic fractions of all tomato products were able to affect model reactions, whatever reactive oxygen species and catalysts were used to drive oxidation. In the XOD/xanthine system both the hydrophilic and lipophilic fractions displayed an inhibitory activity. The hydrophilic fractions were more effective (I_{50} ranging from 680 to 3200 μg, dry weight) than the lipophilic fractions (I_{50} ranging from 4000 to 7750 μg, dry weight). In the MPO/NaCl/H_2O_2 system the hydrophilic fractions inhibited oxidation (I_{50} ranging from 2300 to 2900 μg, dry weight), whereas the lipophilic fractions had a lower inhibitory effect at the same concentration. Conversely, in the copper-catalyzed lipid peroxidation only the lipophilic fractions were effective (I_{50} ranging from 1030 to 2100 μg, dry weight), whereas the hydrophilic fractions had a pro-oxidant effect in the same concentration range. The extent of inhibition varied according to the tomato sample in the superoxide and hydrogen peroxide generating system and in lipid peroxidation, but was substantially the same in the HClO generating system. Fresh tomato varieties differed considerably in the antioxidant activities of their hydrophilic and lipophilic fractions. Processed tomatoes showed a significantly lower antioxidant activity than fresh tomatoes in their hydrophilic fractions but had a high antioxidant activity in their lipophilic fractions. Because the oxidative reactions produced by the above-mentioned model systems are also involved in the pathogenesis of several chronic diseases, the antioxidant activity of tomato fractions might be related to their in vivo activity. Hence, these measurements may be used for optimizing tomato technologies.

Keywords: *Tomato (Lycopersicon esculentum); antioxidant activity; xanthine oxidase; myeloperoxidase; copper-induced lipid peroxidation*

INTRODUCTION

Increased interest in tomato products has been created by the fact that their consumption has been correlated to a reduced risk of some types of cancer (Franceschi et al., 1994; Clinton et al., 1996; Gerster et al., 1997) and ischemic heart disease (Parfitt et al., 1994). Factors contributing to disease protection are believed to correspond to tomato antioxidants, particularly lycopene and β-carotene, which accumulate in plasma and tissues in relation to dietary intake (Oshima et al., 1996). These components may have a role in vivo in inhibiting reactive oxygen species (ROS) mediated reactions, which have been associated with the initiation and progression of a number of pathological processes (Halliwell and Gutteridge, 1990).

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Pathological processes are known to involve complex mechanisms. Xanthine oxidase (XOD) is one of the main enzymatic sources of ROS in vivo. Although XOD present in normal tissue is a dehydrogenase enzyme that transfers electrons to NAD^+, as it oxidizes xanthine or hypoxanthine to uric acid, under certain stress conditions the dehydrogenase is converted to an oxidase enzyme by oxidation of essential thiol groups or by limited proteolysis. Upon this conversion the enzyme reacts with the same electron donors, but reducing oxygen instead of NAD^+, thus producing superoxide and hydrogen peroxide (Halliwell and Gutteridge, 1990; Hippeli and Elstner, 1997). It has been demonstrated that XOD is involved in the oxidative damage occurring after reperfusion of ischemic tissues, in brain edema and injury, and in vascular permeability changes. Furthermore, XOD serum levels are increased in hepatitis and brain tumors (Chang et al., 1994).

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Another source of strong oxidants in vivo is neutrophil myeloperoxidase (MPO), which catalyzes oxidation of chloride ions by hydrogen peroxide, resulting in hypochloric acid production. The cytotoxicity of this reaction contributes to the killing of bacteria in the host defense system. However, hypochloric acid generated by MPO might also inactivate α_1-antiproteinase and contribute to proteolytic damage of healthy human tissues in inflammatory disease (Halliwell and Gutteridge, 1990; Hippeli and Elstner, 1997).

Transition metal ions are powerful catalysts of oxidative reactions in vivo. Iron is the most likely candidate for promoting oxidative reactions, whereas the occurrence of copper-catalyzed reactions in vivo is controversial (Halliwell and Gutteridge, 1990). In fact, organisms take great care in sequestering transition metal ions. Indeed, this sequestration can be regarded as a contribution to antioxidant defense. However, the release of "free" metal ions (i.e., the release of metal ions in forms able to catalyze oxidative reactions) from sequestered sites can occur as a result of tissue injury by disease, trauma, toxins, and other causes (Halliwell and Gutteridge, 1990; Hippeli and Elstner, 1999). There is strong evidence that, under pathological conditions such as cataracts (Cook and McGaharan, 1986), atherosclerotic lesions (Evans et al., 1995), and diabetes (Hunt, 1996), metal ions are released and can be detected in their free and harmful form.

Lipid peroxidation is reported to occur late in injury processes, rather than being the prime cause of damage. However, there are two human pathologies in which it appears to play a major role. One of these is atherosclerosis; the other is deterioration of the brain and spinal cord that occurs after traumatic and ischemic injury (Halliwell and Gutteridge, 1990). Metal-dependent radical reactions seem to contribute to ischemic and traumatic injuries to the brain or spinal cord, where iron- and copper-binding capacity is low. Activated monocytes, which develop into macrophages within the vessel wall, are believed to play a role in the progression of atherosclerosis by secreting hydrogen peroxide, superoxide anion, hydrolytic enzymes, and factors that can stimulate the proliferation of smooth muscle cells. Conversely, the role of metal ions in atherosclerosis progression has not been identified in vivo, although they could become active after the breakdown of metalloproteins (Halliwell and Gutteridge, 1990).

These and other pathological processes have been reviewed (Elstner, 1990; Diplock et al., 1998; Hippeli and Elstner, 1999). Although the role of the different enzymatic and metal catalysts in specific pathological processes is still being clarified, it has been clearly demonstrated that increased ROS formation is implicated in many human diseases. Therefore, in vitro simulation by model systems of oxidative reactions occurring during the initiation and progression of human disease allows the potential inhibitory role of natural mixtures of dietary antioxidants to be studied.

In the present study hydrophilic and lipophilic antioxidant fractions of tomato products were extracted and assessed for antioxidant content (ascorbic acid, total phenolics, and carotenoids) and for antioxidant activity by three simple model reactions, namely, XOD-mediated reaction, MPO-mediated reaction, and copper-catalyzed lipid peroxidation.

The objectives were (a) to develop a procedure for evaluating the antioxidant activity of fresh tomatoes and tomato derivatives both by model systems involving two of the main enzymatic sources of ROS in vivo and by a lipid peroxidation model system and (b) to assess differences in antioxidant activities among some tomato products. Commercial tomato samples differing in variety and processing were studied to represent a composite pool of products.

MATERIALS AND METHODS

Materials. Linoleic acid, xanthine, α-keto-γ-methiolbutyric acid (KMB), 1-aminocyclopropane-1-carboxylic acid (ACC), human myeloperoxidase (MPO), and 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 Tween 20 (especially purified for membrane research) and xanthine oxidase from cow milk (XOD) were obtained from Boehringer Mannheim. Folin−Ciocalteu reagent and HPLC grade unstabilized tetrahydrofuran (THF) were purchased from BDH. HPLC grade methanol, petroleum ether (PE), and butylated hydroxytoluene (BHT) were supplied by Merck.

Tomato Samples. Ripe fresh tomatoes (*Lycopersicon esculentum*) of Rita and Cencara varieties (indicated as fresh 1 and fresh 2, respectively) were purchased from a local market. Prior to extraction and analysis, fresh tomatoes of the same variety were homogenized by an Omni-mixer (17106 Sorvall Du Pont Instrument) for 2 min. Two brands of commercial tomato puree and paste were purchased from a local supermarket.

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 (Porretta, 1991).

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 Dani 3600 chromatograph with a Chromatopack CR-3B Shimadzu integrator and a deactivated aluminum oxide F1 (80−100 mesh) column (1/8 in. × 200 cm): column temperature, 100 °C; injection temperature, 100 °C; FID temperature, 225 °C.

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

THF/PE Extraction. Fresh tomato homogenate, tomato puree, and tomato paste (0.3 g, dry weight) were 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 (12000*g* at 5 °C for 10 min), and residual solids were re-extracted with 15 mL of THF. The second extract was centrifuged (12000*g* at 5 °C for 10 min). The clarified THF extracts were quantitatively combined 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.

Phosphate Buffer Extraction. Fresh tomato homogenate, tomato puree, and tomato paste (0.3 g, dry weight) were added to 10 mL of 0.1 M potassium phosphate buffer, pH 3.0, or alternatively to 10 mL of 0.1 M potassium phosphate buffer, pH 7.4, and stirred gently under nitrogen at room temperature for 15 min. The extract was centrifuged (12000*g* at 5 °C for 10 min), and residual solids were supplemented with 10 mL of 0.1 M potassium phosphate buffer, pH 7.4. 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. It was then stirred gently under nitrogen at room temperature for 60 min. The second

extract was centrifuged (12000*g* at 5 °C for 10 min), and residual solids were washed with 5 mL of bidistilled water. The clarified extracts were combined, and the total volume was measured. Extracts were stored under nitrogen in the dark at 4 °C. Extractions were carried out in duplicate.

Carotenoids. Carotenoids were determined after extraction with unstabilized THF/PE, as described above, and with stabilized THF/PE (0.1% BHT) according to the method of Khachik et al. (1986). The HPLC procedure of Riso and Porrini (1997) was followed. A Vydac 201TP54 C_{18} column (250 × 4.6 mm), equipped with a C_{18} precolumn, was used. Chromatographic separation was performed with methanol/stabilized THF (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 detected using a UV−vis detector set at 454 nm.

Ascorbic Acid. Ascorbic acid was determined after extraction with phosphate buffer as described above and with 5% metaphosphoric acid/1 mM EDTA (instead of diethylenetriaminepentaacetic acid) according to the method of Prior et al. (1998). The HPLC procedure of Mannino and Pagliarini (1988) was followed. 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 versus Ag/AgCl.

Total Phenolics. Total phenolics were determined after phosphate buffer extraction, THF/PE extraction as described above, and 50% methanol/water extraction according to the method of Vinson et al. (1998). Analysis was performed by visible spectrophotometry at 760 nm, after reaction with Folin−Ciocalteu reagent. Dried aliquots of THF/PE extract were redissolved in 50% methanol/water and filtered with a 0.22 μm filter (carotenoids did not dissolve in this solvent). Phosphate buffer and methanol/water extracts were purified as reported by Di Stefano and Cravero (1991). Extracts (1 mL) were diluted with water (1 mL) and 1 N H_2SO_4 (2 mL), and 2 mL of this solution 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 with 2 mL of 0.1 N H_2SO_4 prior to phenolics elution with 2 mL of methanol and 5 mL of distilled water. Results were calculated by a calibration curve obtained from chlorogenic acid.

Antioxidant Activity. The antioxidant activity of phosphate buffer and THF/PE extracts was evaluated. Dried aliquots of THF/PE extracts were dissolved in acetone prior to addition to the XOD/xanthine and MPO/NaCl/H_2O_2 systems and in THF/10% Tween 20 (1:10) prior to addition to the linoleic acid/$CuSO_4$ system. Phosphate buffer extracts were filtered with a 0.45 μm filter. Extracts from fresh tomatoes were also ultrafiltered by a Millipore membrane with a 5 kDa cutoff to exclude possible interference due to the presence of enzymes. However, ultrafiltration was found to be necessary only for the linoleic acid/$CuSO_4$ system.

XOD/Xanthine System. This system contained 0.1 M phosphate buffer, pH 7.4, 0.5 mM xanthine (in 10 mM NaOH), 0.04 unit of XOD, 1.25 mM KMB, and various extract concentrations in phosphate buffer or acetone (the final acetone concentration was 2.5%). The reaction was carried out at 37 °C for 30 min, followed by GC to measure ethene release from KMB (Kruedener et al., 1995).

MPO/NaCl/H_2O_2 System. This system contained 0.1 M phosphate buffer, pH 6.0, 150 mM NaCl, 0.1 mM H_2O_2, 0.025 unit of MPO, 1.25 mM ACC, and various extract concentrations in phosphate buffer or acetone (the final acetone concentration was 5%). The reaction was carried out at 37 °C for 30 min, followed by measurement of ethene release from ACC (Kruedener et al., 1995).

Linoleic Acid/$CuSO_4$ System. This system contained 0.05 M phosphate buffer, pH 7.0, 1% Tween 20, 1.2 mM linoleic acid, 5 μM $CuSO_4$, and various extract concentrations in phosphate buffer or THF/10% Tween 20, 1:10 (the final THF concentration was 0.6%). The reaction was carried out at 37 °C for 60 min, followed by UV−vis spectrophotometry. The formation of conjugated dienes of hydroperoxides (absorbance at 234 nm) was determined to test the THF/PE extracts, whereas the thiocyanate method was used for the phosphate buffer extracts because they interfered with UV absorption. The procedure of Yen and Hsieh (1998) was modified as follows. Hydroperoxides were determined by sequentially adding 75% ethanol (3.7 mL), 30% ammonium thiocyanate (1 mL), an aliquot of the reaction mixtures (0.75 mL), and freshly prepared 0.02 M ferrous chloride in 3.5% HCl (0.1 mL). After 3 min, the absorbance (500 nm) of the mixtures was recorded.

Control reactions were prepared for all model systems by adding the solvent (phosphate buffer, acetone, or THF/10% Tween 20, 1:10) instead of the antioxidant solution. Trolox was used as a positive control in all model systems. In the XOD/xanthine and MPO/NaCl/H_2O_2 systems identical dose−response curves for Trolox were obtained both in the presence and in the absence of acetone. In the linoleic acid/$CuSO_4$ system identical dose−response curves for Trolox were obtained by measuring hydroperoxides by conjugated dienes and by the thiocyanate reaction. The antioxidant activity was calculated as percent of inhibition of the control reaction rate and expressed both as I_{50}, as interpolated by the dose−response curves, and as Trolox equivalents. I_{50} is the amount of sample (micrograms, dry weight) that caused 50% inhibition of model reaction, in the reaction volume, under the conditions described. Trolox equivalents are the ratio of the I_{50} of Trolox (nanomoles) to the I_{50} of the sample (milligrams, dry weight).

Data Expression. All results were obtained from a minimum of four independent experiments, and the relevant means were calculated. Analyses of variance were performed by ANOVA procedures. Significant differences ($P < 0.05$) between means were determined by Duncan's multiple-range test. Data were expressed on a dry weight basis.

RESULTS AND DISCUSSION

Extraction of Hydrophilic and Lipophilic Antioxidant Fractions. The extractability of water-soluble antioxidants of tomato was assessed by a two-step extraction using either 0.1 M phosphate buffer, pH 7.4, in both steps or 0.1 M phosphate buffer, pH 3.0, and 0.1 M phosphate buffer, pH 7.4, sequentially (Table 1). The latter procedure increased ascorbic acid and total phenolics recoveries probably because both compounds were more stable at acid pH. This procedure was compared with previously published methods performed on the same sample (Table 1): ascorbic acid was extracted with 5% metaphosphoric acid according to the method of Prior et al. (1998), and phenolics were extracted with 50% methanol/water at 90 °C according to the method of Vinson et al. (1998). The agreement of the results between our procedure and the reference procedures was satisfactory for both unconjugated phenolics and ascorbic acid recovery.

Lipid-soluble antioxidants of tomato were extracted with THF. The use of THF for carotenoid extraction has been introduced by Khachik et al. (1986) and found to be very effective for quantitative analysis (Tonucci et al., 1995; Riso and Porrini, 1997). According to this method, BHT (0.1%) is added to the extracting solvent to prevent carotenoid oxidation during extraction. However, because BHT interfered with the evaluation of the antioxidant activity, a procedure for extracting lipophilic antioxidants from tomato samples was developed by using THF without the addition of external antioxidants, followed by purification with PE. Our procedure was compared with the procedure of Khachik performed on the same sample (Table 2). In fresh tomatoes the extraction yield of the proposed procedure for β-carotene was 10% lower than that of the reference procedure;

Table 1. Comparison among Different Procedures for Ascorbic Acid and Total Phenolics Extraction from Fresh Tomatoes[a]

extraction solvent	ascorbic acid (μg/g, dry wt)	total phenolics (μg/g, dry wt)
proposed procedures		
(a) 0.1 M phosphate buffer, pH 7.4 (first step)	1300 ± 30	3800 ± 20
0.1 M phosphate buffer, pH 7.4 (second step)	150 ± 20	490 ± 30
first + second extracts	1450 ± 50[a]	4290 ± 50[a]
(b) 0.1 M phosphate buffer, pH 3.0 (first step)	1400 ± 10	3890 ± 10
0.1 M phosphate buffer, pH 7.4 (second step)	200 ± 10	750 ± 10
first + second extracts	1600 ± 20[b]	4640 ± 20[b]
reference procedures		
(c) 5% metaphosphoric acid, 1 mM EDTA[b]	1700 ± 10[c]	nd[d]
(d) 50% methanol/water[c]	nd	4550 ± 20[b]

[a] Values bearing different superscripts in a column are significantly different ($P < 0.05$). [b] Prior et al. (1998). [c] Vinson et al. (1998). [d] Not determined.

Table 2. Comparison between Two Procedures for Carotenoid Extraction from Fresh Tomatoes[a]

extraction solvent	lycopene (μg/g, dry wt)	β-carotene (μg/g, dry wt)	lutein (μg/g, dry wt)
proposed procedure			
unstabilized THF/PE	1160 ± 30[a]	161 ± 5[a]	16 ± 2[a]
reference procedure			
stabilized THF/PE (0.1% BHT)[b]	1230 ± 20[a]	178 ± 2[b]	18 ± 1[a]

[a] Values bearing different superscripts in a column are significantly different ($P < 0.05$). [b] Khachik et al. (1986).

however, yields for lycopene and lutein were not statistically different.

These results indicated that the proposed extraction procedures for hydrophilic and lipophilic fractions of tomato provided good antioxidant yields, as compared to the reference procedures. The proposed procedures should cause little or no changes in the natural antioxidant pool under milder conditions (i.e., room temperature under nitrogen atmosphere) in the absence of external stabilizing and chelating agents.

Antioxidant Content. The tomato products analyzed in this study were assessed for ascorbic acid, carotenoid, and total phenolic contents (Table 3). The two fresh tomato varieties had very different ascorbic acid contents. Ascorbic acid was lower in tomato derivatives than in fresh products, especially tomato paste, probably as a result of the severity of heat treatment. In a previous study the ascorbic acid content was found to be an index of heat damage of tomato during drying (Zanoni et al., 1999). The two fresh tomato varieties also showed different carotenoid contents. High lycopene and β-carotene contents were observed in heat-treated tomatoes, whereas lutein was not detectable. Indeed, heat treatments (pasteurization, stewing, and drying) have been found to cause little damage to the lycopene and β-carotene content of tomato (Dietz and Gould, 1986; Khachik et al., 1992; Tonucci et al., 1995; Zanoni et al., 1999). The total phenolics solubilized in phosphate buffer were similar for all of the samples analyzed, with slightly higher values for tomato paste. Values obtained from fresh tomato samples are in accordance with those reported by Vinson et al. (1998). A fraction of total phenolics was also found in the THF/PE extract. Interestingly, the two fresh tomato varieties had an almost opposite antioxidant content: the ascorbic acid content of the Rita variety (fresh 1) was almost twice and the carotenoid content was almost half that of the Cencara variety (fresh 2). On the other hand, the two varieties had similar phenolic contents.

Antioxidant Activity. The XOD/xanthine system produces hydrogen peroxide and superoxide and decomposes KMB, releasing ethene (Kruedener et al., 1995). Both the hydrophilic and lipophilic fractions of all tomato samples inhibited KMB fragmentation by XOD. Dose−response curves are reported in Figures 1 and 2. I_{50} and Trolox equivalent values were calculated as described under Materials and Methods and are reported in Table 4. Hydrophilic fractions were shown to have a higher antioxidant activity than lipophilic fractions. The hydrophilic fractions of the two fresh tomato varieties showed marked differences in antioxidant activity, and fresh samples had a significantly higher

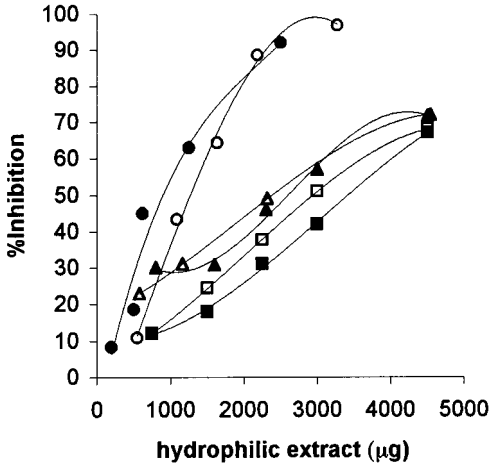

Figure 1. Inhibitory effect of hydrophilic extracts from fresh tomato (●, ○), tomato puree (■, □), and tomato paste (▲, △) on KMB fragmentation by the XOD/xanthine system (as percent of the control reaction rate).

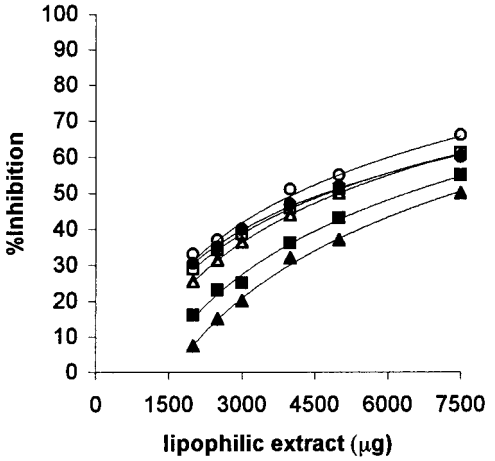

Figure 2. Inhibitory effect of lipophilic extracts from fresh tomato (●, ○), tomato puree (■, □), and tomato paste (▲, △) on KMB fragmentation by the XOD/xanthine system (as percent of the control reaction rate).

Table 3. Antioxidant Content of Some Commercial Tomato Products[a]

		antioxidant content (μg/g, dry wt)					
		phosphate buffer extract		THF/PE extract			
sample	total solids (%)	ascorbic acid	total phenolics	lycopene	β-carotene	lutein	total phenolics
fresh tomato 1	6.2 ± 0.2	3050 ± 100^{e}	3330 ± 300^{a}	740 ± 40^{a}	56 ± 2^{ab}	5.4 ± 0.2^{ab}	422 ± 50^{a}
fresh tomato 2	5.88 ± 0.05	1550 ± 10^{d}	4640 ± 40^{b}	1160 ± 40^{c}	161 ± 10^{d}	16 ± 5^{b}	650 ± 100^{ab}
tomato puree 1	10.3 ± 0.03	850 ± 10^{c}	4600 ± 50^{b}	1560 ± 20^{d}	76 ± 2^{c}	0^{a}	792 ± 70^{b}
tomato puree 2	11.55 ± 0.01	902 ± 10^{c}	4400 ± 40^{b}	960 ± 20^{b}	43 ± 5^{a}	0^{a}	420 ± 70^{a}
tomato paste 1	22.3 ± 0.1	530 ± 6^{b}	4800 ± 50^{b}	1540 ± 20^{d}	78 ± 5^{c}	0^{a}	753 ± 30^{b}
tomato paste 2	31.6 ± 0.2	337 ± 1^{a}	5560 ± 30^{c}	1510 ± 20^{d}	66 ± 5^{bc}	0^{a}	795 ± 60^{b}

[a] Values bearing different superscripts in a column are significantly different ($P < 0.05$).

Table 4. Antioxidant Activity of Hydrophilic and Lipophilic Fractions of Tomato Products As Measured by the XOD/Xanthine System[a,b]

	XOD/xanthine system			
	hydrophilic fraction		lipophilic fraction	
sample	I_{50} (μg)	Trolox equiv (nmol/mg)	I_{50} (μg)	Trolox equiv (nmol/mg)
fresh tomato 1	680 ± 7^{a}	750 ± 20^{c}	4400 ± 200^{a}	117 ± 8^{c}
fresh tomato 2	1290 ± 60^{b}	395 ± 30^{b}	4000 ± 200^{a}	128 ± 9^{c}
tomato puree 1	3200 ± 160^{d}	160 ± 10^{a}	6300 ± 600^{b}	81 ± 10^{ab}
tomato puree 2	3000 ± 30^{d}	170 ± 5^{a}	4800 ± 350^{a}	106 ± 10^{bc}
tomato paste 1	2450 ± 60^{c}	210 ± 10^{a}	7750 ± 380^{c}	66 ± 5^{a}
tomato paste 2	2350 ± 90^{c}	220 ± 14^{a}	5000 ± 450^{ab}	100 ± 11^{bc}

[a] Values bearing different superscripts in a column are significantly different ($P < 0.05$). [b] The I_{50} value for Trolox was 510 ± 10 nmol.

Table 5. Antioxidant Activity of Hydrophilic Fractions of Tomato Products As Measured by the MPO/NaCl/H_2O_2 System[a,b]

	MPO/NaCl/H_2O_2 system, hydrophilic fraction	
sample	I_{50} (μg)	Trolox equiv (nmol/mg)
fresh tomato 1	2900 ± 60^{b}	530 ± 20^{a}
fresh tomato 2	2500 ± 30^{a}	610 ± 20^{a}
tomato puree 1	2300 ± 100^{a}	670 ± 80^{a}
tomato puree 2	2300 ± 100^{a}	670 ± 50^{a}
tomato paste 1	2400 ± 120^{a}	640 ± 50^{a}
tomato paste 2	2300 ± 70^{a}	670 ± 60^{a}

[a] Values bearing different superscripts in a column are significantly different ($P < 0.05$). [b] The I_{50} value for Trolox was 1530 ± 30 nmol.

antioxidant activity than processed samples, as expected in view of the differences in ascorbic acid contents. However, a significant antioxidant activity was also found in tomato pastes with a low ascorbic acid content, probably due to the antioxidant activity of phenolic components. Less marked differences were observed in the antioxidant activity of the lipophilic fraction. Some authors attributed the antioxidant activity of phenolics to their inhibitory effect on XOD (Meyer et al., 1995). Our previous study on the measurement of oxygen consumption during XOD-catalyzed reaction in the presence of tomato extract demonstrated that, in the concentration range used, no direct inhibition of XOD occurred (Lavelli et al., 1999). Therefore, under our conditions, the inhibitory effect of tomato extracts should be attributed to an ROS scavenging action rather than to XOD inhibition.

The MPO/NaCl/H_2O_2 system produces hypochloric acid, which specifically fragments ACC, resulting in ethene formation (Kruedener et al., 1995). The hydrophilic fractions of all tomato products inhibited ACC fragmentation by MPO. Dose−response curves and I_{50} and Trolox equivalent values are reported in Figure 3 and Table 5, respectively. Very flat dose−response curves were obtained for the lipophilic fractions, which did not allow us to make an accurate calculation of the I_{50}. The lipophilic fractions were markedly less effective than the hydrophilic fractions, as their Trolox equivalent values were by 1 order of magnitude lower (~56 nmol/mg). Slight differences were found in the antioxidant activities of tomato products. As discussed for the XOD/xanthine system, the decrease in ethene release occurring in the presence of antioxidant extracts can be attributed to scavenging of ROS and/or to direct inhibition of the MPO catalyst by some phenolic components (Volpert and Elstner, 1996).

Table 6. Anti- and Pro-oxidant Activity of Hydrophilic and Lipophilic Fractions of Tomato Products As Measured by the Linoleic Acid/$CuSO_4$ System[a,b]

	linoleic acid/$CuSO_4$ system		
	hydrophilic fraction	lipophilic fraction	
sample	% increase in peroxidation rate with 2000 μg extract	I_{50} (μg)	Trolox equiv (nmol/mg)
fresh tomato 1	56 ± 7^{c}	2100 ± 20^{d}	3.5 ± 0.1^{a}
fresh tomato 2	30 ± 3^{b}	1030 ± 10^{a}	7.1 ± 0.2^{d}
tomato puree 1	16 ± 1^{a}	1135 ± 55^{b}	6.4 ± 0.4^{cd}
tomato puree 2	14 ± 2^{a}	1360 ± 5^{c}	5.4 ± 0.1^{b}
tomato paste 1	14 ± 1^{a}	1097 ± 20^{ab}	6.7 ± 0.2^{cd}
tomato paste 2	15 ± 1^{a}	1190 ± 25^{b}	6.1 ± 0.2^{bc}

[a] Values bearing different superscripts in a column are significantly different ($P < 0.05$). [b] The I_{50} value for Trolox was 7.3 ± 0.1 nmol.

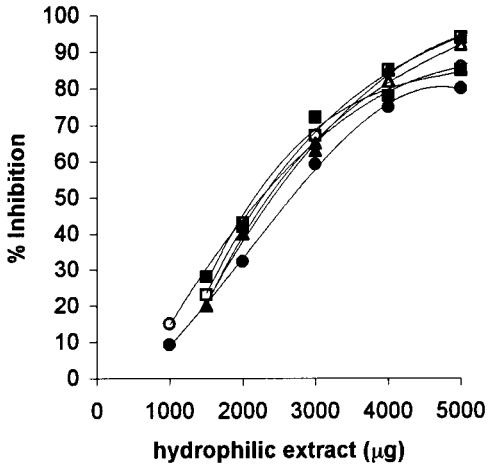

Figure 3. Inhibitory effect of hydrophilic extracts from fresh tomato (●, ○), tomato puree (■, □), and tomato paste (▲, △) on ACC fragmentation by the MPO/NaCl/H_2O_2 system (as percent of the control reaction rate).

The linoleic acid/copper system was used as a lipid peroxidation model. The hydrophilic fractions of all tomato samples had a pro-oxidant effect on the copper-catalyzed linoleic acid oxidation. Table 6 shows the percent increase in peroxidation rate in the presence of 2 mg (dry weight) of hydrophilic extracts. This effect

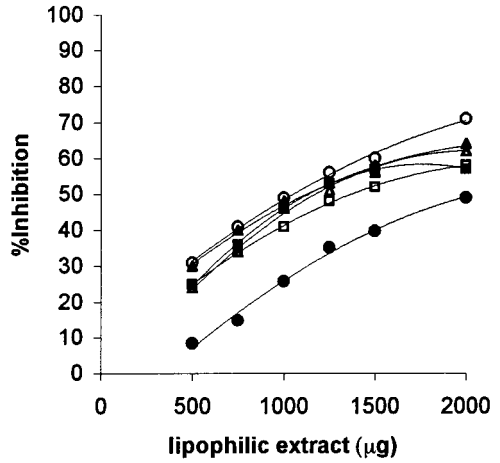

Figure 4. Inhibitory effect of lipophilic extracts from fresh tomato (●, ○), tomato puree (■, □), and tomato paste (▲, △) on conjugated dienes of hydroperoxides formation by the linoleic acid/$CuSO_4$ system (as percent of the control reaction rate).

may be due to the presence of ascorbic acid, which has been reported to activate metal ion-catalyzed reactions (Halliwell and Gutteridge, 1990). Indeed, the prooxidant effect was highest in samples with the highest ascorbic acid content. In contrast, the lipophilic fraction had an inhibitory effect, as shown by the dose−response curves in Figure 4. Marked differences were found in I_{50} values between the fresh tomato varieties, whereas a slight difference was observed between the processed products (Table 6). In this system the highest antioxidant activity was found in the samples with the highest carotenoid contents.

The first aim of this study was to assess the antioxidant activity of fresh and processed tomatoes by different model reactions. It is not surprising that the measurement of antioxidant activity of biological samples depended on which free radical or oxidant was used in the assay. Elstner (1990) reviewed different biochemical models to study the antioxidant properties of plants used in phytotheraphy. Other authors used different chemical and biochemical models to assess the antioxidant activity of common vegetables and tea (Cao et al., 1996; Hagerman et al., 1998; Yen and Hsieh, 1998). Therefore, a comparison between various test reactions as indicator systems for antioxidant activity measurements allows more accurate information to be obtained. Moreover, it has been observed that the antioxidant activity depends on both concentrations and synergistic effects. Interactions between antioxidants may bring about effects that are not observed when individual constituents are tested (Diplock et al., 1998). For example, synergistic effects have been identified between antioxidants such as ascorbic acid and some low molecular weight thiols (glutathione and dihydrolipoate) and α-tocopherol, as well as between β-carotene and α-tocopherol (Sies and Stahl, 1995).

As far as the properties of fresh tomato are concerned, in earlier studies the radical scavenging activity of hydrophilic and lipophilic fractions was studied either by using β-phycoerythrin as a target for free radical damage and a synthetic hydrophilic radical generator, 2,2′-azobis(2-amidinopropane) dihydrochloride, as a catalyst (Wang et al., 1996) or by measuring directly the interaction with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS) (Pellegrini et al., 1999). The hydrophilic fraction of tomato was found to have a higher antioxidant activity than the lipophilic fraction in both systems.

In the present study tomato antioxidant properties were assessed by using the same model systems that have been proven to be useful to test extracts from plants used in phytotheraphy such as *Populus tremula*, *Solidago virgaurea*, and *Fraxinus excelsior* (Meyer et al., 1995). It was demonstrated that tomato extracts could inhibit oxidation catalyzed by two of the main sources of ROS in vivo, namely, XOD and MPO, involving hydrogen peroxide, superoxide, and hypochloric acid. Our previous study showed that hydrophilic tomato extracts also inhibited peroxynitrite-catalyzed reaction (Lavelli et al., 1999). More complex information was obtained from lipid peroxidation models. Previously, methanolic tomato extract was found to inhibit copper-catalyzed oxidation of lower density lipoproteins (LDL + VLDL) isolated from plasma (Vinson et al., 1998). Methanolic tomato extract was also found to inhibit iron ascorbate-induced lipid peroxidation of microsomes enriched with specific cytochrome P 450 isoenzymes (Plumb et al., 1997). The water fraction of tomato exhibited inhibitory properties against rat liver microsome lipid peroxidation induced by CCl_4 but promoted β-carotene oxidation in a β-carotene/linoleic acid emulsion (Gazzani et al., 1998a,b). The lipophilic fraction, extracted from tomato, containing carotenoids was not very effective on the XOD/xanthine and MPO/NaCl/H_2O_2 systems but very effectively inhibited the copper-catalyzed lipid peroxidation. This behavior was contrary to that of the hydrophilic fraction. Altogether, hydrophilic and lipophilic tomato extracts show antioxidant activities toward a variety of ROS that can be generated in vivo. Therefore, tomato products have the potential capability to address the prevention of oxidative damage in vivo.

The second aim of this study was to assess differences in antioxidant activity among tomato products, which have been scarcely investigated in earlier studies. This is of fundamental importance to the optimization of tomato technologies. In our previous study ripening conditions were shown to affect greatly both the antioxidant accumulation kinetics and the final antioxidant content of tomato (Giovanelli et al., 1999). In the present study statistically significant ($P < 0.05$) differences were found in the antioxidant activity of tomato products according to the variety and processing. Although experiments were carried out on a very small number of samples, the following two general conclusions may be drawn: (a) The antioxidant activity of tomato varies considerably according to the variety; hence, this aspect of quality can be improved by genetic selection. (b) A considerable loss of hydrophilic antioxidant fraction, particularly ascorbic acid, is caused by the technological process. This loss should be minimized through process optimization.

ABBREVIATIONS USED

ACC, 1-aminocyclopropane-1-carboxylic acid; BHT, butylated hydroxytoluene; KMB, α-keto-γ-methiolbutyric acid; MPO, myeloperoxidase; PE, petroleum ether; THF, tetrahydrofuran; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; XOD, xanthine oxidase.

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