Properties of Tomato Powders As Additives for Food Fortification and Stabilization

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The antioxidant activities of two freeze-dried tomato powders as additives for food fortification and stabilization were studied. The two tomato powders were obtained from the whole fruit and from the pulp after "serum" separation, respectively. The antioxidant activity was studied by measuring (a) the inhibition of the singlet oxygen-catalyzed oxidation of α-linolenic acid, in the presence or absence of copper ions, as a model of the oxidative processes occurring in foods, and (b) the inhibition of xanthine oxidase (XOD)- and myeloperoxidase (MPO)-catalyzed reactions and copper-induced lipid peroxidation. The partial separation of "serum" decreased the freeze-drying time by 50%. The partially fractionated tomato powder had a 60% lower phenolic content and an 11-fold higher lycopene content than the whole tomato powder, on a dry weight basis. Ascorbic acid was almost completely removed by fractionation. Both the powder obtained from the whole tomato and that obtained from the partially fractionated tomato had antioxidant activity in all the model systems used. Based on these results, we conclude that tomato powders have multifunctional properties, which could address the prevention of oxidative degradations both in foods and in vivo. Therefore, tomato can be regarded as source of food additives for fortification and stabilization, even if it is submitted to technological processes that can cause the loss of the more labile hydrophilic antioxidants.

Keywords: *Tomato (Lycopersicon esculentum); singlet oxygen quenching; free radical scavenging; copper chelation*

INTRODUCTION

Natural antioxidants have been recognized to exhibit crucial roles both in vivo and in foods. The first role is related to the antioxidants' potential ability to prevent relevant disease processes through the inhibition of oxidative damage to biological macromolecules caused by reactive oxygen species (ROS). This health-protective role is exhibited in human plasma and tissues, where the antioxidant level increases as a function of dietary intake (*1*−*3*). The second role is related to the antioxidant involvement in the maintenance of food nutritional and sensory quality, as these components can inhibit oxidative degradations during processing and storage of foods (*4*, *5*).

Biological tissues are continuously subjected to oxidative stress from different ROS produced by numerous chemical reactions and biochemical pathways. In our previous works we studied the potential health-protective role of plant antioxidants through the simulation of various oxidative reactions that occur during the initiation and progression of human diseases. Such reactions were driven by the main biological catalysts producing ROS, namely, xanthine oxidase (XOD), myeloperoxidase (MPO), several NAD(P)H dehydrogenases (diaphorases, DIAs), and by transition metal ions (*6*−*9*). In the fore-mentioned studies we demonstrated that plants used in phytotherapy (such as *Salix*, *Propolis*, *Fraxinus*, *Populus*, and *Solidago*) and dietary components (i.e., tomato products) share similar abilities to inhibit different biochemical model reactions.

Similar to the antioxidant defense system in vivo, a multicomponent, multiphasic antioxidant system is necessary to control the oxidative reactions that occur in foods. The antioxidant function in foods is mainly related to the following three mechanisms: (a) transition metal ion chelation, (b) scavenging of peroxyl and alcoxyl radicals, deriving from unsaturated fatty acid oxidation, and (c) quenching of singlet oxygen, generated through light activation of sensitizers (such as chlorophyll, riboflavin, and heme-containing protein) to an excited state, which, in turn, can transfer their energy to oxygen (*4*). All these antioxidant mechanisms can be examined using a model reaction developed by Heiser et al. (*10*). According to this model reaction, peroxidation of α-linolenic acid is catalyzed by photodynamic activation of rose bengal, in the presence or absence of copper ions. Therefore, the antioxidant activity can be studied as the ability to scavenge free radicals, and/or to quench singlet oxygen produced by the photoactivated sensitizer rose bengal, and/or to chelate copper.

Owing to their antioxidant properties, different vegetables and fruits can be regarded as interesting sources of functional additives for food fortification and stabilization. Indeed, some studies have dealt with the use of aromatic herbs as additive in oils or oil-in-water emulsions (*11*, *12*). For this use, antioxidant mixtures which are stable under food processing conditions are required. Tomato carotenoids have been found to be stable under severe oxidative and thermal process

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conditions (*13*−*16*). On the other hand, the hydrophilic tomato antioxidants were greatly affected by processing (*16*, *17*).

The aim of the present work was to study the potential properties as food additives of two freeze-dried tomato powders which were obtained from the whole fruit and from the pulp after "serum" separation, respectively.

MATERIALS AND METHODS

Materials. α-Linolenic acid, linoleic acid, xanthine, α-keto-γ-methiolbutyric acid (KMB), 1-aminocyclopropane-1-carboxylic acid (ACC), rose bengal (RB), human myeloperoxidase (MPO), and reference samples for *all-trans* lycopene (90−95% purity), ascorbic acid (99.0% purity), and chlorogenic acid (95% purity) were obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. 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 (Mannheim, Germany). Folin−Ciocalteu reagent and HPLC-grade unstabilized tetrahydrofuran (THF) were purchased from BDH (Zug, Zwitzerland). HPLC-grade methanol and petroleum ether (PE) were supplied by Merck (Darmstadt, Germany).

Freeze-Dried Tomato Powders. For each dehydration trial, 5 kg of fresh tomatoes (*Lycopersicon esculentum*) were used. Fruits were washed, heated in hot water for 3 min, peeled, deprived of parenchyma and seeds and homogenized using, sequentially, a 1-mm and a 0.5-mm sieve. The juice obtained was separated into two aliquots (750 g each). One aliquot was freeze-dried. The second aliquot was centrifuged (12 000*g* at 5 °C for 15 min); the supernatant ("serum", about 80% w/w) was eliminated and the precipitate was added to 600 mL of 1% citric acid. After 30 min of equilibration at room temperature, the mixture was centrifuged (12 000*g* at 5 °C for 15 min). The supernatant was discarded again and the precipitate was freeze-dried. For freeze-drying a Lyoflex Edwards (Crawley, UK) apparatus was used. The final moisture content of the powders was in the range of 5−10%.

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 (*18*).

pH and Titratable Acidity. The pH and titratable acidity were determined as described previously (*19*), after re-hydration of 1.5 g of the powders with 40 mL of distilled water.

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 and a deactivated aluminum oxide column (1/8 in. x 100 cm): column temperature, 60 °C; injection temperature, 80 °C; FID temperature, 225 °C.

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

Extractions of the Hydrophilic and Lipophilic Antioxidant Fractions. For the extraction of the hydrophilic antioxidants, tomato powder (0.3 g, dry weight) was added to 20 mL of 0.1 M potassium phosphate buffer, pH 7.4, stirred gently under nitrogen at room temperature for 60 min, and then centrifuged (12000*g* at 5 °C for 10 min). For the extraction of the lipophilic antioxidants, THF and PE were used according to the procedure described in Lavelli et al. (*9*). Because THF interfered with the GC analysis, tomato powder was also extracted with acetone: the powder from the whole or the partially fractionated fruit (0.8 and 0.08 g dry weight, respectively) was added to 20 mL of acetone, stirred gently under nitrogen at room temperature for 60 min, and then centrifuged (12000*g* at 5 °C for 10 min). Preliminary experiments showed that phosphate buffer provided a good extraction yield for phenolics (≥94%), and THF/PE and acetone extractions provided good extraction yields for lycopene (≥98%). Extraction yields were assayed according to the procedure described in Lavelli et al. (*9*).

Antioxidant Content. Antioxidant content was analyzed as described previously (*9*). Briefly, carotenoids were determined by HPLC equipped with an UV−vis detector; ascorbic acid was analyzed by HPLC coupled with an electrochemical detector; and total phenolics was determined by the Folin-Ciocalteau reaction using chlorogenic acid as a standard.

Antioxidant Activity. The antioxidant activity was evaluated by the following model systems.

α-Linolenic Acid/RB and α-Linolenic Acid/RB/$CuSO_4$ Systems. The α-linolenic acid/RB system contained 0.1 M phosphate buffer, pH 7.4, 3.55 mM α-linolenic acid (dissolved as described in *10*), 20 μM RB, and various extract concentrations in phosphate buffer or acetone (the final acetone concentration was 10%). In the α-linolenic acid/RB/$CuSO_4$ system, 3 μM $CuSO_4$ was added. The reactions were carried out in tubes sealed with gastight rubber stoppers, for 30 min at 37 °C, in the light (500 $\mu E/m^2 \times s$), and followed by GC to measure ethane and ethene release (*10*).

XOD/Xanthine System. This system contained 0.1 M phosphate buffer, pH 7.4, 0.5 mM xanthine (in 10 mM NaOH), 0.08 unit of XOD, 1.25 mM KMB, and various extract concentrations in phosphate buffer. The reaction was carried out in tubes sealed with gastight rubber stoppers for 30 min at 37 °C, and followed by measurement of ethene release from KMB (*20*).

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 units of MPO, 1.25 mM ACC, and various extract concentrations in phosphate buffer. The reaction was carried out in tubes sealed with gastight rubber stoppers at 37 °C for 30 min, and followed by measurement of ethene release from ACC (*20*).

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 THF/10%Tween 20, 1:10. The reaction was carried out for 60 min at 37 °C, and followed by UV spectrophotometry (at 234 nm) to measure the formation of conjugated dienes of hydroperoxides.

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. The antioxidant activity was calculated as % of inhibition of the control reaction rate. For each extract a minimum of four dilutions were analyzed in quadruplicate.

RESULTS AND DISCUSSION

Tomato fractionation by centrifugation allowed a partial separation of "serum" (80% w/w) and decreased the freeze-drying time by 50%. To prevent spoilage by microorganisms, after centrifugation the pellet was washed by citric acid, and a final titratable acidity around 10% was obtained (Table 1), which falls in the range of the titratable acidity of fresh tomato and tomato concentrates (*21*).

The antioxidant content and the antioxidant activity of the freeze-dried powders were evaluated on the same extracts, therefore a direct comparison between the composition of the powders and their functionality can be made. It is worth nothing that the extractions of the hydrophilic and the lipophilic fractions were carried out in the absence of external antioxidants to avoid interference with the measurement of antioxidant activity. By this approach, a loss in the ascorbic acid content is unavoidable, however, good recoveries of phenolics and lycopene can be obtained.

As shown in Table 1, the lipophilic extract of the partially fractionated powder had an 11-fold higher lycopene content on a dry weight basis as compared to that of the corresponding extract obtained from the

Table 1. Characterization of the Tomato Powders Obtained from the Whole Fruit and after Partial "Serum" Separation[a]

characteristic (unit)	whole tomato	partially fractionated tomato
pH	4.3 ± 0.1	3.4 ± 0.1
acidity (g citric acid/ 100 g, dry wt)	8.13 ± 0.4	10.5 ± 0.3
antioxidant content		
ascorbic acid (mg/kg, dry wt)[b]	1460 ± 20	92 ± 5
total phenolics (mg/kg, dry wt)[b]	4133 ± 200	1600 ± 150
lycopene (mg/kg, dry wt)[b]	474 ± 45	5399 ± 600

[a] Data are expressed as average ± SD ($n = 2$). [b]Antioxidants were extracted without adding stabilizing agents, as for antioxidant activity evaluation.

Table 2. Control Runs for the α-Linolenic Acid/RB and the α-Linolenic Acid/RB/$CuSO_4$ Model Systems: α-Linolenic Acid Peroxidation (Ethane and Ethene Release) As Catalyzed by Photodynamic Activation of RB, in the Presence or in the Absence of Copper Ions and Acetone

conditions	reaction rate (nmol/mL*min)[a] ethane	reaction rate (nmol/mL*min)[a] ethene	ethane/ethene molar ratio
− $CuSO_4$ − acetone	57 ± 4	0.59 ± 0.04	97 ± 9
+ $CuSO_4$ − acetone	188 ± 10	82 ± 3	2.3 ± 0.2
− $CuSO_4$ + acetone	83 ± 6	1.02 ± 0.03	81 ± 8
+ $CuSO_4$ + acetone	350 ± 10	152 ± 12	2.3 ± 0.3

[a] Data are expressed as average ± SD ($n = 4$).

whole fruit. In the hydrophilic extract of the partially fractionated tomato powder a part of the polar antioxidants of tomato was still present: phenolic and ascorbic acid contents were respectively 2.6- and 16-fold lower as compared to those of the corresponding extract obtained from the whole fruit.

First, the antioxidant activity of the hydrophilic and lipophilic fractions of the whole and the partially fractionated tomato powders was studied by using the α-linolenic acid/RB and α-linolenic acid/RB/$CuSO_4$ reactions developed by Heiser et al. (*10*) as models for oxidative processes occurring in foods. These reactions can be followed by measuring ethane and ethene release. As shown in Table 2, in the absence of $CuSO_4$, the main product of α-linolenic acid peroxidation was ethane; in fact, the ethane/ethene molar ratio was around 90. In the presence of the metal catalyst, the production of ethane and, above all, that of ethene, was greatly increased. Besides the Cu^{2+}-mediated lipid peroxidation, these results can be explained by the Cu^{2+}-mediated oxidation of ethyl radical, which derives from the (*ω*-3)-end of α-linolenic acid via peroxidation, forming ethene (*22*):

$$Cu^{2+} + {}^{\bullet}CH_2{-}CH_3 \rightarrow Cu^{+} + H^{+} + CH_2{=}CH_2$$

The α-linolenic acid peroxidation rate was also increased by the presence of acetone, which was used to solubilize the lipophilic tomato extracts. However, acetone did not affect the ethane/ethene molar ratio (Table 2).

Components acting as free radical scavengers or singlet oxygen quenchers can inhibit both the α-linolenic acid/RB and the α-linolenic acid/RB/$CuSO_4$ model systems. In addition, α-linolenic acid/RB/$CuSO_4$ can also be inhibited by copper chelators. The measurement of ethane to ethene conversion can be used to assess copper chelating activity (*8*).

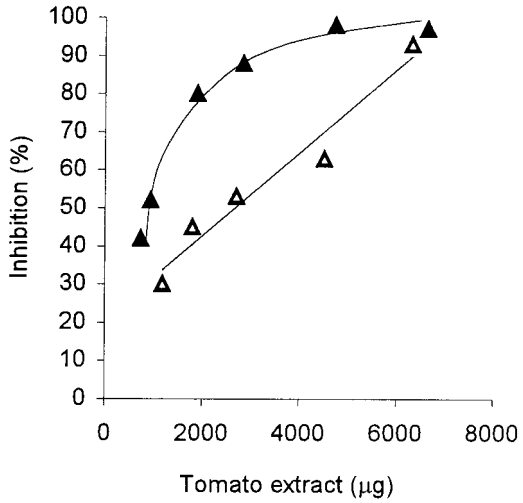

Figure 1. Inhibitory effect of the hydrophilic extracts of freeze-dried tomato powders obtained from the whole (▲) and partially fractionated (Δ) fruit, on RB-catalyzed α-linolenic acid peroxidation (as percent of control reaction rate measured by ethane + ethene release). The variation coefficient of the experimental values was in the range 3−7%.

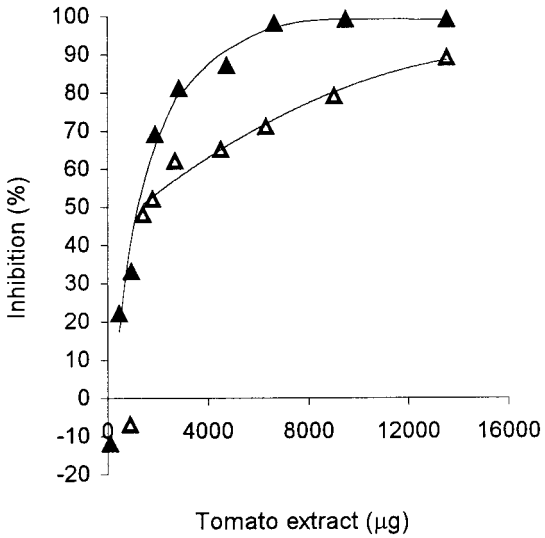

Figure 2. Inhibitory effect of the hydrophilic extracts of freeze-dried tomato powders obtained from the whole (▲) and partially fractionated (Δ) fruit, on RB- and $CuSO_4$-catalyzed α-linolenic acid peroxidation (as percent of control reaction rate measured by ethane + ethene release). The variation coefficient of the experimental values was in the range 2−5%.

The antioxidant activity of the hydrophilic extracts of the tomato powders, as measured by the α-linolenic acid/RB and the α-linolenic acid/RB/$CuSO_4$ model systems, is shown in Figures 1 and 2, respectively. As expected, the hydrophilic extract of the whole tomato product had a higher antioxidant activity than that of the partially fractionated product, in both these model systems, which is consistent with the higher phenolic and ascorbic acid contents. In the α-linolenic acid/RB model system, the I_{50} values were 910 ± 40 and 2700 ± 20 μg for the extract obtained from the whole tomato powder and the extract obtained from the partially fractionated tomato powder, respectively. In the α-linolenic acid/RB/$CuSO_4$ model system, low concentrations of the extracts of both products had a pro-oxidant activity. On increasing extract concentration a sharp increase in the antioxidant activity was observed for both extracts, which had similar I_{50} values but reached

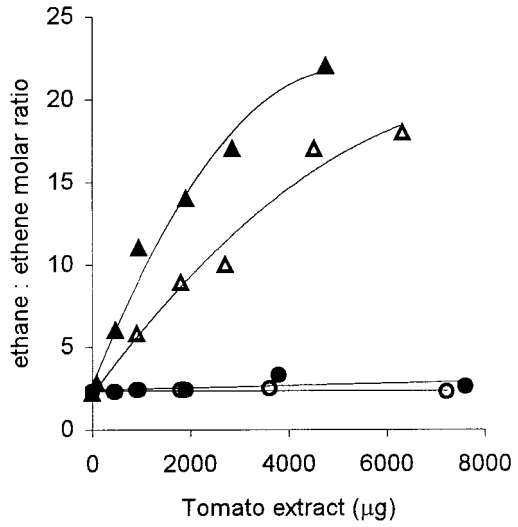

Figure 3. Ethane/ethene molar ratio produced by RB- and $CuSO_4$-catalyzed α-linolenic acid peroxidation, in the presence of the following extracts of tomato powders: hydrophilic extracts of the whole (▲) and partially fractionated (Δ) fruit, and lipophilic extracts of the whole (●) and partially fractionated (○) fruit.

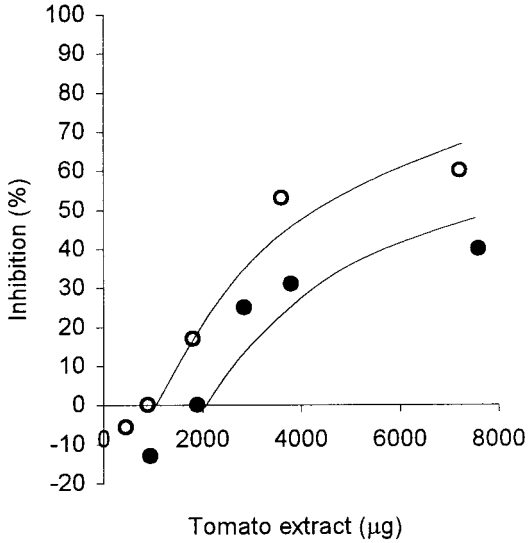

Figure 4. Inhibitory effect of the lipophilic extracts of freeze-dried tomato powders obtained from the whole (●) and partially fractionated (○) fruit, on RB-catalyzed α-linolenic acid peroxidation (as percent of control reaction rate measured by ethane + ethene release). The variation coefficient of the experimental values was in the range 3−8%.

the maximum level of inhibition at about 4800 and 13 000 μg, respectively. As shown in Figure 3, both tomato products inhibited the ethane to ethene conversion; i.e., they formed an inactive complex with copper ions. The partially fractionated tomato powder inhibited this conversion to a lesser extent, despite a higher citric acid content (as indicated in Table 1). Again, this behavior could be ascribed to its lower content of phenolics, some of which are known to act as copper chelators (*23*).

The antioxidant activity of the lipophilic extracts of the tomato powders as measured by the α-linolenic acid/RB and the α-linolenic acid/RB/$CuSO_4$ model systems, is reported in Figures 4 and 5. The extracts showed a similar inhibition trend and the antioxidant activity of the partially fractionated product was about double that of the whole tomato product, in both these model

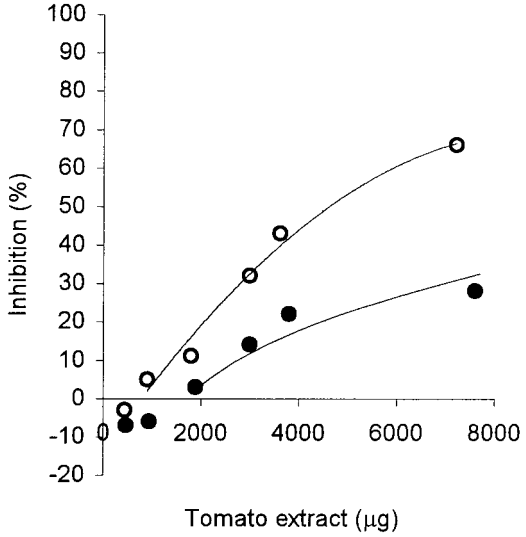

Figure 5. Inhibitory effect of the lipophilic extracts of freeze-dried tomato powders obtained from the whole (●) and partially fractionated (○) fruit, on RB- and $CuSO_4$-catalyzed α-linolenic acid peroxidation (as percent of control reaction rate measured by ethane + ethene release). The variation coefficient of the experimental values was in the range 3−8%.

systems, despite the 11-fold increase in lycopene content. As shown in Figure 3, neither the extract obtained from the whole tomato fruit nor that obtained from the partially fractionated fruit affected the ethane to ethene conversion, i.e., the lipophilic extracts did not chelate copper ions. The lipophilic extracts had a moderate pro-oxidant effect on lipid peroxidation at low extract concentrations, both in the presence and in the absence of copper ions. A pro-oxidant effect of carotenoids has been observed by different authors under certain experimental conditions (*24*), and could be ascribed to carotenoid photooxidation. It has been suggested that during the oxidative degradation of β-carotene, the carotenoid may also participate in free radical reactions and possibly enhance the degradation rates of either the carotenoid itself or the accompanying unsaturated lipid (*25*).

The antioxidant activity of tomato powders was then studied using the MPO/NaCl/H_2O_2, XOD/xanthine, and linoleic acid/$CuSO_4$ model systems, which simulate some oxidative processes occurring in vivo.

The antioxidant activity of the hydrophilic fractions of the tomato powders as measured by the MPO/NaCl/H_2O_2 and XOD/xanthine model systems, is reported in Figures 6 and 7. In the MPO/NaCl/H_2O_2 model system the extract obtained from the whole tomato powder and that obtained from the partially fractionated tomato powder had I_{50} values of 3750 ± 50 and 6070 ± 50 μg, respectively. In the XOD/xanthine system, the partially fractionated tomato powder had much lower antioxidant activity than the whole tomato powder.

The antioxidant activity of the lipophilic fractions of the tomato powders as measured by the linoleic acid/$CuSO_4$ model system, is reported in Figure 8. The extract obtained from the whole tomato powder and that obtained from the partially fractionated tomato powder had I_{50} values of 3480 ± 40 and 870 ± 30 μg, respectively, which again were not consistent with their lycopene content.

The use of antioxidant additives for food stabilization and fortification will be particularly effective where they possess multifunctional properties. Therefore, in the

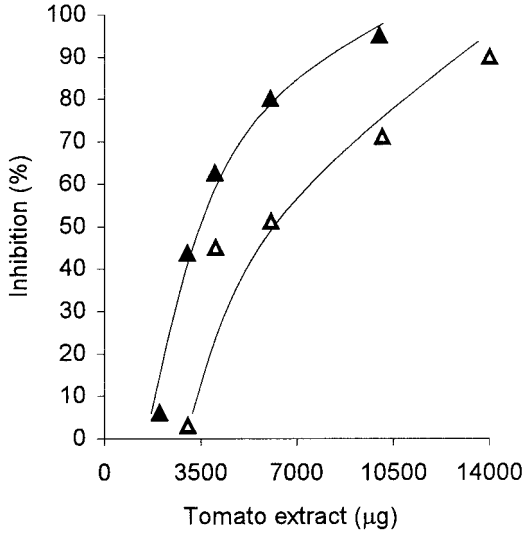

Figure 6. Inhibitory effect of the hydrophilic extracts of freeze-dried tomato powders obtained from the whole (▲) and partially fractionated (Δ) fruit, on ACC fragmentation by MPO (as percent of control reaction rate measured by ethene release). The variation coefficient of the experimental values was in the range 2−4%.

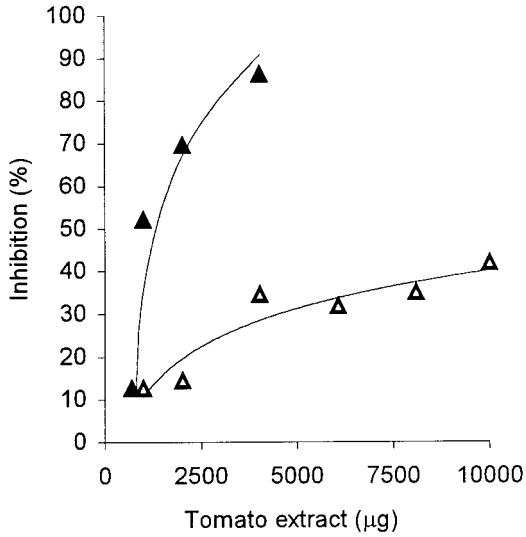

Figure 7. Inhibitory effect of the hydrophilic extracts of freeze-dried tomato powders obtained from the whole (▲) and partially fractionated (Δ) fruit, on KMB fragmentation by XOD (as percent of control reaction rate measured by ethane + ethene release). The variation coefficient of the experimental values was in the range 1−4%.

present study the antioxidant activity of tomato powders was investigated using a lipid peroxidation model reaction, driven by light and copper ions. It was particularly crucial to verify the interaction of antioxidant extracts with copper ions, which accelerate lipid peroxidation by hydrogen abstraction and peroxide decomposition, resulting in the formation of free radicals. Cao et al. (*26*) found that different vegetable extracts acted as antioxidants when copper was used as an oxidation catalyst; conversely, single antioxidants such as ascorbic acid and α-tocopherol acted as pro-oxidants in the presence of the transition metal ions. Indeed, many natural compounds are capable of chelating metals, however, some chelators inhibit oxidation, whereas others accelerate oxidative reactions through different mechanisms as reviewed by Decker (*4*). An additional catalyst of lipid peroxidation of relevant importance in food is singlet oxygen, which

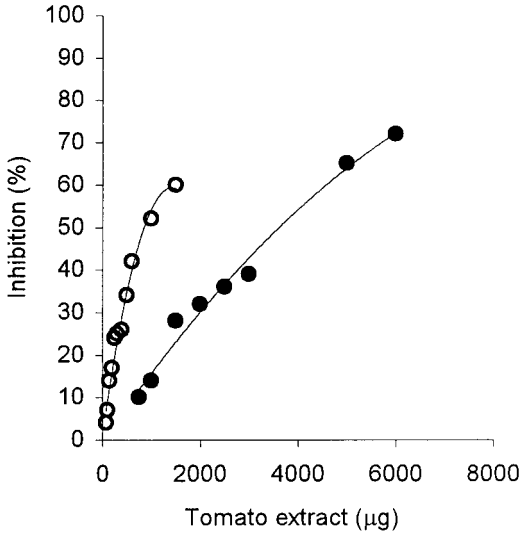

Figure 8. Inhibitory effect of the lipophilic extracts of freeze-dried tomato powders obtained from the whole (●) and partially fractionated (○) fruit, on $CuSO_4$-catalyzed linoleic acid peroxidation (as percent of control reaction rate measured by hydroperoxide conjugate dienes formation). The variation coefficient of the experimental values was in the range 1−5%.

tomato products are expected to be able to inactivate because of their content of carotenoids. In fact, carotenoids can physically quench the excited state of oxygen; in particular, lycopene, which is peculiar to tomato, was found to be the most efficient singlet oxygen quencher (*27*). In the present study, by using a model of oxidative reactions occurring in foods, we found that the hydrophilic antioxidant fraction of tomato powders had both scavenging/quenching activities and copper chelating activity, whereas the lipophilic antioxidant fraction of tomato had only scavenging/quenching activities. The addition of natural antioxidants to foods also leads to increased potential health benefit. In addition to the inhibition of lipid peroxidation, we found that the tomato powders could inhibit XOD- and MPO-catalyzed reactions, which represent relevant oxidative processes occurring in vivo.

On comparing the partially fractionated tomato powder with the whole tomato powder, we observed that the former had a lower antioxidant activity in the hydrophilic solvent and a higher antioxidant activity in the lipophilic solvent, in all model systems used. The antioxidant activity of the lipophilic fraction did not show a linear relationships with respect to lycopene content. This behavior could be ascribed to the loss of phenolic compounds, which during fractionation were partitioned both in the serum and in the pulp. A part of tomato phenolic compounds is present in the lipophilic extract (*9*) and could significantly contribute to its antioxidant activity. In fact, Vinson et al. (*28*) have shown that phenolic compounds were much more effective as antioxidants toward the copper-catalyzed LDL oxidation than the carotenoids, ascorbic acid, and α-tocopherol. In addition, fractionation could have abolished possible synergistic actions between antioxidant components, which have been discussed by various authors (*2, 29, 30*).

On the other hand, to successfully develop antioxidant additives for food stabilization and fortification it is also crucial to take into account the effect of processing on antioxidant content. It is well-known that tomato carotenoids are little affected by processing (*13*−*16*) but

ascorbic acid is degraded to various extents. Accordingly, the antioxidant activity of tomato lipophilic fraction, as measured by the linoleic acid/$CuSO_4$ model system was retained, whereas the antioxidant activity of tomato hydrophilic fraction, as measured by the XOD/xanthine system, decreased upon oxidative heat treatment (*16*, *17*). Therefore, the lipophilic antioxidant fraction of tomato should be considered as the main promising source of antioxidant additives.

ABBREVIATIONS USED

ACC, 1-aminocyclopropane-1-carboxylic acid; BHT, butylated hydroxytoluene; DIA, diaphorase; KMB, α-keto-γ-methiolbutyric acid; MPO, myeloperoxidase; PE, petroleum ether; RB, rose bengal, THF, tetrahydrofuran; XOD, xanthine oxidase.

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