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Potent Antioxidative Activity of Lycopene: A Potential Role in Scavenging Hypochlorous Acid †

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Abstract

Lycopene, a carotenoid found in tomatoes, is a proven anti-oxidant that may lower the risk of certain disorders including heart disease and cancer. Hypochlorous acid (HOCl) is an oxidant linked to tissue oxidation in cardiovascular disease and other inflammatory disorders through its ability to modify proteins, deoxyribonucleic acid, ribonucleic acid and lipids. Here we show that lycopene can function as a potent scavenger of HOCl at a wide range of concentrations that span various pathophysiological and supplemental ranges. The oxidation of lycopene by HOCl was accompanied by a marked change in color, from red to colorless, of the lycopene solution suggesting lycopene degradation. HPLC and LC-MS analysis showed that the exposure of lycopene to increasing concentrations of HOCl gave a range of metabolites resulting from oxidative cleavage of one or more C=C. The degree of degradation of lycopene (as assessed by the number and chain lengths of the different oxidative metabolites of lycopene) depends mainly on the ratio between HOCl to lycopene, suggesting that multiple molecules of HOCl are consumed per molecule of lycopene. Collectively, this work demonstrates a direct link between lycopene and HOCl scavenging, and may assist in elucidating the mechanism of the protective function exerted by lycopene.

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Keywords

Lycopene; myeloperoxidase; hypochlorous acid; carotenoid oxidation

Introduction

Lycopene, the red pigment of tomato, is a tetraterpene assembled from eight isoprene units, composed entirely of carbon and hydrogen containing 11 conjugated and 2 non-conjugated carbon-carbon double bonds (C=C) (Figure 1). It is one of the most interesting unsaturated carotenoids with well-known health benefits [1,2]. Lycopene concentration in human serum tends to be higher than all other carotenoids pigments [2]. Increasing evidence suggests that lycopene could be involved in protection against chronic disorders such as cardiovascular disease, prostate cancer, respiratory and digestive epithelial cancers [3–7]. Lycopene exerts potent anti inflammatory effects through its action as an antioxidant and free radical scavenger, which may reduce cellular damage [8,9]. It also plays an important role in protecting cell membranes from lipid peroxidation, neutralizing hydroxyl radicals and may bind to DNA, promoting further protection beyond antioxidant activity. Additionally, lycopene may stimulate antioxidative enzymes such as superoxide dismutase, glutathione peroxidase, and glutathione reductase [10], as well as inhibit H₂O₂-induced lipid peroxidation and lipoprotein modification [11]. The mechanism by which lycopene exerts these antioxidant properties *in vivo* has not yet been elucidated.

Lycopene metabolites generated through lycopene oxidation may have different activities than lycopene or can display completely new and independent biological functions [12]. Indeed, studies from several laboratories have demonstrated potential beneficial effects of lycopene specifically in response to oxidants. These beneficial functions include enhanced cellular gap junction communication, induction of phase II enzymes through activation of the antioxidant response element (ARE) transcription system, suppression of insulin-like growth factor-1-stimulated cell proliferation by induction of insulin-like growth factor binding protein, anti-angiogenesis, inhibition of cell proliferation, and induction of apoptosis [12,13].

HOCl and its conjugate base (OCl⁻) are potent oxidants that function as powerful antimicrobial agents. [14]. However, the properties of HOCl that makes it such a potent antimicrobial agent may also endanger the host, as it can damage the host tissue by the same mechanism used to destroy invading pathogens [15]. HOCl is generated enzymatically by myeloperoxidase (MPO), a neutrophil derived heme peroxidase, which uses hydrogen peroxide (H₂O₂) produced during respiratory burst to catalyze the two electron oxidation of chloride (Cl⁻). HOCl is implicated as a contributing factor in a number of pathological conditions including inflammatory diseases, atherosclerosis, respiratory distress, acute vasculitis, rheumatoid arthritis, glomerulonephritis and cancer [16–20]. Recently, we showed that HOCl plays an important role in accelerating oocyte aging through a mechanism that involves oocyte fragmentation and degradation [21]. Different types of biological molecules react with HOCl. For example, NADH and NH-groups of pyrimidines nucleotides are chlorinated by a HOCl-induced reaction. HOCl causes extensive denaturation of double-stranded DNA [22, 23]. HOCl can convert tyrosine residues in a protein to form 3-chlorotyrosine [24]. In lipids the major site of attack by HOCl are the double bonds of unsaturated fatty acids and cholesterol, leading to either chlorohydrin formation [24] or peroxidation [25, 26]. In addition to being a potent oxidant itself, HOCl can react with other compounds to produce other reactive oxidant species (ROS) and free radicals. For example, HOCl can react with superoxide (O₂^{•-}) to generate hydroxyl radical (•OH) [27,28]. In alkaline solution hypochlorite, the conjugate base of HOCl reacts with

H₂O₂ to yield singlet oxygen species [29]. Importantly, these ROS and free radicals subsequently generated from HOCl may cause further cellular damage.

MPO and its downstream final products have recently been used as markers for many chronic disorders. Consequently, inhibition of MPO and/or eliminating its end product, HOCl, is a topic of great interest in several laboratories. More recently, we have shown that melatonin, tryptophan, and tryptophan analogues display the potential capacity to reversibly inhibit MPO through the accumulation of MPO Compound II (MPO-Fe(IV)=O), an inactive MPO intermediate [30–32]. The aim of this work is to highlight the potential role of lycopene as a pathway of HOCl scavenging, to identify the HOCl-mediated oxidative products of lycopene, and their cleavage mechanism. Identification of potent scavenger of HOCl from dietary supplements may provide novel avenues for preventing initiation, progression and development of inflammation-related pathological states.

Materials and Methods

Materials

All the materials used were of highest purity grade and used without further purification. Lycopene was obtained from Toronto Research Chemicals Inc. (ON, Canada). Sodium hypochlorite (NaOCl), tetrahydrofuran (THF), ammonium acetate (CH₃COONH₃), taurine, and 5, 5-Dithiobis (2-nitro-benzoic acid) were obtained from Sigma Aldrich (St. Louis, MO, USA). HPLC grade methanol, acetonitrile (CH₃CN), isopropanol and chloroform were obtained from Acros (Belgium).

Spectral analysis

Optical spectra were recorded on a Cary 100-Bio UV-visible spectrophotometer at 23°C. Lycopene was prepared as 4 mM stock solution dissolved in chloroform and stored at –80 °C until further use. Care was taken not to expose lycopene solution to light, either during stock solution preparation or experimentation. To measure the effect of HOCl on lycopene destruction, 12.5 µl aliquots of the lycopene stock solution were removed and diluted to 500 µl CHCl₃ in Pyrex test-tubes, and the lycopene solutions then received equal volume of aqueous solution (500 µl) containing increasing concentration of HOCl (0–3750 µM). The solution mixtures were vortexed continuously for 2 min in the dark and the aqueous layer was removed. 50 µl from the organic phase was diluted to 1 ml in CHCl₃ and the absorbance spectra were scanned from 200 to 700 nm.

High Performance Liquid Chromatography (HPLC) analysis

HPLC analyses was carried out using a Shimadzu HPLC system equipped with a SCL-10A system controller, with a binary pump solvent delivery (LC-10 AD) module and a SIL-10AD auto-injector connected to a SPD-M10A diode array detector (DAD). Alltech 5 µm particle size, 4.6 × 150 mm reverse-phase octadecylsilica (C18) HPLC column was used. The column was kept at 27 °C. The photodiode array detector was set at 450 nm to obtain the chromatogram. The gradient system used was a slight modification of a previously published method [33]. After oxidation with HOCl, the chloroform layer was evaporated under a stream of nitrogen and then the dried sample was re-suspended in the injection solvent (25 µl CHCl₃ and 975 µl CH₃CN) solution, and 50 µl were injected. The column was eluted at a flow rate of 1.0 mL/min with linear gradients of solvents A and B (A, CH₃CN: THF: 1% CH₃COONH₃ in H₂O = 50:20:30, v/v/v; B, CH₃CN: THF: 1% CH₃COONH₃ in H₂O = 50:44:6, v/v/v). The solvent gradient used were, A-70 %, B-30% at start, then a linear gradient increase of B to 70 % in 70 min, then a steeper gradient to increase B to 100 % in 1 min and hold at 100 % B for 10 min. At the end of the run the system was equilibrated with 100 % solvent A. Under these conditions, lycopene eluted at

around 46 min and was detected from the characteristic spectral signal from the Diode Array Detector. Each sample was analyzed in triplicate.

Mass spectrometric analysis

Mass spectrometry (MS) experiments were performed using an Agilent 6410 Triple Quadrupole mass spectrometer coupled with an Agilent 1200 HPLC system (Agilent Technologies, New Castle, DE), equipped with a multimode source. Alltech Prevail C18 column (particle size 5 μ m; 4.6 $\times$ 150 mm; Nicholasville, KY) was used to separate reaction products. Solvent A was methanol and solvent B was isopropanol. The column was equilibrated with 80% solvent A and 20% solvent B. The gradient was: 20–50% solvent B over 12 min; 50% solvent B for 2 min; 50–20% solvent B for 1 min; and 80% solvent A for 15 min. 2 μ L of the reaction mixture was injected at a flow rate of 1 mL/min. Liquid chromatography atmospheric pressure chemical ionization (LC/APCI) MS in the positive and negative modes were performed using the following parameters: spray voltage 2500 V, drying gas flow 5 L/min, drying gas temperature 350°C, vaporizer temperature 150°C, and nebulizer pressure 20 psi. Flow injection analysis (FIA) was used to optimize the fragmentor voltage. Optimal fragmentor voltage for lycopene was 130 V in MS2 scan mode. Mass range between m/z 100 and m/z 700 was scanned to obtain full scan mass spectra.

Solution preparation

HOCl preparation—HOCl was prepared following a slight modification of a published method [34]. Briefly, a stock solution of HOCl was prepared by adding 7.5 ml NaOCl solution to 10 ml potassium phosphate (KH_2PO_4) solution (100 mM). The concentration of HOCl in this stock solution was determined by the taurine chloramine assay [35]. As HOCl is unstable, the stock solution was freshly prepared on a daily basis, stored on ice, and used within one hour of preparation. For further experimentations, dilutions were made from the stock solution using 100 mM KH_2PO_4 , to give working solutions of lower HOCl concentration.

Results

HOCl-promotes lycopene oxidation

Lycopene, a member of the carotenoid pigment family, possesses a characteristic red color. Lycopene, like all carotenoids, has the ability to absorb light in the 400–500 nm region of the visible spectrum. We initially studied the reaction of HOCl with lycopene to understand the role of lycopene in HOCl scavenging, and to isolate and identify lycopene cleavage products. Reactions were carried out at 25°C in chloroformic solution. From our initial experiments we observed the disappearance of lycopene color to determine whether HOCl promotes its oxidation. The oxidation of lycopene by a slight excess of HOCl was accompanied by a marked change from red color to colorless (Fig. 2, inset). The color disappeared rapidly, and after 2 min a complete loss of the red color of lycopene occurred. Fig. 2 compares the absorbance spectra of lycopene exposed to increasing concentration of HOCl with that of untreated lycopene. The visible spectrum of lycopene displayed characteristic peak centered at 460, 486, and 520 nm in chloroform. Exposure of a fixed amount of lycopene to increasing concentration of HOCl caused lycopene exhaustion, as indicated by the flattening, shift, and disappearance of its characteristic absorbance peaks. Lycopene is an unsaturated hydrocarbon with 11 $\text{C}=\text{C}$, of which 11 double bonds are conjugated and the characteristic UV-visible spectra of lycopene is due to this hyperconjugated double bonds in the molecule (Fig. 1). The disappearance of lycopene spectra could be due to the loss of hyperconjugation in the molecule and/or lycopene fragmentation.

HPLC analysis of lycopene oxidation products

As HOCl is thought to oxidize carotenoids, we examined if these spectral transformations that is apparent from our UV-visible spectral analysis may represent the oxidation and subsequent fragmentation of lycopene by HOCl. HPLC analysis was performed (as mentioned in the materials and methods section), to study the reaction products after oxidation of lycopene with HOCl. We incubated a fixed amount of lycopene (50 μ M) with increasing HOCl concentrations (250, 500, 625 μ M). When lycopene was reacted with HOCl there was a progressive reduction in the lycopene signal (as a function of HOCl concentration) along with the formation of new peaks eluting at earlier time, Fig. 3A shows the chromatograms for control and the different reaction mixtures at 450 nm. In our method lycopene eluted at 47 min and was identified by its characteristic spectra observed from the photodiode array detector. Calculating the area under the curve for lycopene revealed that in a sample treated with 250 μ M of HOCl 12% of lycopene was remaining; while in 500 μ M of HOCl 0.5% of lycopene was remaining. Complete loss of the lycopene peak was observed in 625 μ M of HOCl (Fig. 3B). The fact that there is residual lycopene remaining at 500 μ M of HOCl proves that one molecule of lycopene is capable of scavenging multiple molecules of HOCl. The appearance of newer earlier eluting peaks in the chromatograms, could be due to the formation of new compounds with shorter chain lengths and subsequently lower hydrophobicity.

LC/APCI/MS analysis of lycopene oxidation products

LC/APCI/MS was utilized to elucidate whether HOCl-mediated reaction products of lycopene, were indeed due to oxidation and fragmentation of the lycopene molecule. To optimize the MS parameters 100 pmol of lycopene was first analyzed by flow injection analysis (FIA) method at different fragmentor voltages in APCI positive and negative ionization modes. Optimal fragmentor voltage for lycopene was determined to be 130 V in MS2 scan mode. Several solvent systems were evaluated to obtain maximal ion intensity of MS detector. Ammonium acetate and tetrahydrofuran suppressed ion intensity. Therefore, we chose isopropanol because this solvent has the same polarity with tetrahydrofuran and does not significantly suppress ionization. Methanol and isopropanol gradient as described in Methods gave the best ion intensity (data not shown).

The predominant species of lycopene metabolite in the chloroformic solution with lycopene: HOCl of 1:5 was eluted at 2.86 min, and identified as m/z 393 in the APCI positive mode. The extracted ion chromatogram for m/z 393 and the mass spectrum of this compound is depicted in Fig. 4A and 4B respectively. The second major peak was observed at 1.75 min and identified as m/z 349. This compound contains 2 carbonyl groups and represents double oxidative modifications (Fig 5 and Table 1). Incubation of lycopene with 1:10 and 1:12.5 ratios gave a range of metabolites. In the sample with a ratio of 1:10 (lycopene to HOCl) a series of long chain compounds were obtained with m/z values of 301, 393, 413, 437, 441, 469 and 475 and elution times of 2.05, 2.86, 2.69, 1.85, 2.96, 3.18 and 1.78 min, respectively. In contrast, after the incubation of lycopene with higher concentration of HOCl/OC1 (1:15 and 1:20) all lycopene was consumed, and traces of m/z 301 was still detectable. In addition, the chloroformic solution with a ratio of 1:15 showed the formation of two new metabolites with elution times of 2.26 and 2.38 min with m/z values of 329 and 421, respectively, while the solution of a ratio of 1:20 led to the generation of three new metabolites: a) m/z 443; elution time 1.86 min; b) m/z 443; elution time 2.14 min; and c) m/z 449; elution time 2.03 min. The extracted ion chromatogram of m/z 443 is depicted in Fig 6A showing the 2 elution times for this mass indicating presence of two distinct compounds. Examination of the mass spectrum (Fig 6B) of the first compound reveals presence of one chlorine atom as the ion intensity of $[M + H + 2]^+$ ion is approximately 40% of $[M + H]^+$ indicating a chlorine isotope pattern. In contrast, the isotopic pattern of m/z 443 from the

second peak has no intense $[M + H + 2]^+$ ion (Figure 6C) indicating that this product is not chlorinated. In the negative ion mode, peaks were observed for m/z values of 255, 281, 283, 409, 437 and 473 with elution times of 2.12, 2.07, 2.40, 2.50, 2.80, and 4.12 min respectively.

The majority of the products were tentatively identified by detecting the corresponding molecular weight and comparing the proposed structures with the previously identified products, and/or due to the chemical reactivity of HOCl with carotenoids [36]. Identification of the eluted reaction products identified the presence of three type of lycopene oxidation. The first group contains lycopene metabolites resulting from single oxidative cleavage. These metabolites contain on one end an acid and/or an aldehyde group, and on the other end of the metabolite the Ψ -end group of lycopene, and are called apo-lycopenal and apo-lycopenoic acid. In the positive APCI mode, the m/z 393 (Figure 4) and m/z 443 (Fig 6C) are likely produced by single oxidative cleavage.

The second group contains lycopene metabolites resulting from a double oxidative cleavage. This category of lycopene metabolites contain two acid end groups and are called apo-carotendials, because their structure could have been obtained from any carotenoid and not only from lycopene. The m/z 349 (Fig. 5) represents such a compound.

The third group contains other compounds resulting from further chlorination of group 2 and is called chloro-apo-carotendial. The m/z 443 (Fig 6B; retention time 1.86 min) represents a chlorinated lycopene metabolite.

Discussion

Increased levels of HOCl, a potent oxidant, are typically observed in the plasma and tissues of individuals with inflammatory diseases [37]. And yet, a functional deficiency of taurine, a potent HOCl scavenger, is a defining feature of diabetes, obesity, depression, hypertension, gout, kidney failure, and autism, among other conditions [38, 39]. Previous epidemiological evidence has suggested that the intake of lycopene is associated with a reduced risk for many chronic disorders including prostate cancer and heart diseases [40]. Although most investigators have attributed the potential protective role of lycopene in the prevention of chronic diseases to its antioxidant function [41], the exact mechanism by which lycopene exerts these antioxidant effects have not been fully elucidated. Using a combination of direct UV-visible, HPLC and LC/APCI/MS, we have shown that increasing concentration of HOCl can alter the availability of lycopene through a mechanism that involves lycopene oxidation and fragmentation. Exposure of lycopene to saturated amounts of HOCl caused a distinct bleaching in color suggesting lycopene destruction. HPLC and LC-MS analysis showed that exposure of lycopene to increasing concentrations of HOCl gave a range of metabolites resulting from the oxidative cleavage of one or more C=C. HOCl may directly mediate the destruction of lycopene by unselective cleavage at any double bond position in a nonenzymatic manner. HOCl-mediated lycopene oxidation was rapid in chloroformic solution, and a total of thirteen different cleavage products have been identified based on their mass signals through the treatment of lycopene solution with a range of HOCl (1:5 to 1:20 molar ratio). The degree of degradation of lycopene (as assessed by the number and chain lengths of the different oxidative metabolites of lycopene) depends mainly on the ratio between HOCl to lycopene, suggesting that multiple molecules of HOCl are consumed per molecule of lycopene. Thus, HOCl interaction with lycopene may serve as a potential mechanism for modulating its availability, and thereby influencing the regulation of local inflammatory and infectious events *in vivo*.

These cleavage products can be classified into three major categories according to the degree of oxidation and their cleavage position within the lycopene molecule: the first group contains lycopene metabolites resulting from single oxidative cleavage. These metabolites contain on one end an aldehyde/or an acid group and on the other end of the metabolite the Ψ-end group of lycopene, and were called apo-lycopenal and apo-lycopenoic acid. Indeed, two apo-lycopenal and two apo-lycopenoic acids were obtained and tentatively identified based on the m/z value. Apo-lycopenal isolated fragments showed that the double bonds 5–6 (or 5′–6′), was affected, whereas apo-lycopenoic acid showed that the double bonds 13–14 (or 13′–14′), 15–15′ and 9–10 (or 9′–10′) were affected by the cleavage. All the apo-lycopenoic acid fragments (acycloretioic acid, apo-14′-lycopenoic acid, and apo-10′-lycopenoic acid) were identified at both low (1:5) and high (1:20) lycopene to HOCl ratio. Apo-lycopenal fragments (apo-6′-lycopenal) were identified at only higher lycopene to HOCl ratio (Table 1). The second group resulting from the oxidation and modification of the both end groups of lycopene that could occur at position 1–2 (or 1′–2′) or at any other position to produce lycopene metabolites with: two acids, two aldehydes, or combination of both acid and aldehyde on the two end groups and was called apo-carotendials, because their structure could have been obtained from any carotenoid and not only from lycopene. In case of apocarotendials where the 1–2 (or 1′–2′) in one side and the other side showed that the double bonds (7–8) (or 7′–8′) were affected whereas the corresponding acids showed that the double bonds 7–8 (or 7′–8′), 11–12 (or 11′–12′) and 5–6 (or 5′–16′) were affected by the cleavage. In addition, among the different products isolated, three fragments were oxidatively modified at both ends of lycopene's hyperconjugated chain. These three products were crocetin semialdehyde, crocetin, and apo-5′, 6′ carotendial (Table 1). The third group contains other compounds resulting from further chlorination of lycopene metabolites, called chloro-apo-carotendials. This includes m/z 443 which is a chlorinated lycopene fragment: 2 hydroxy 3 chloro 4,6,8,10,12,14,16,18 docosotene 1,22 diol 6,11,15,19 tetramethyl. Thus, taken together; the results in our studies suggest that the HOCl-mediated cleavage reaction of lycopene and the degree of oxidation could occur unselectively at any position of the lycopene double bonds independent of HOCl concentration. The formation of long-chains was not detected in higher ratio of lycopene to HOCl (e.g., 1:30, data not shown), suggested that the detected fragments are further oxidized to shorter-chain lengths upon increasing the HOCl concentration.

In the case of potassium permanganate-mediated generation of apo-carotendials, Caris-Veyrat *et al.* have shown that only the double bonds 5–6 (or 5′–6′), 7–8 (or 7′–8′), 9–10 (or 9′–10′), and 11–12 (or 11′–12′) were affected by the cleavage. But they did not detect apo-carotendials/ones resulting from cleavages of double bonds closer to the center of the molecule (15–15′ C=C) [44]. No cleavage was detected on the double bond which is the farthest from the center of the molecule, i.e., the 1–2 double bond, which is not conjugated and is trisubstituted. The author attributed this behavior to the non-reactivity of these positions to the oxidation with potassium permanganate [44]. No products resulting from a double oxidative cleavage (apo-carotendial) were detected upon exposure of lycopene to the oxidative catalytic species *trans*-dioxoruthenium(VI)-tetraphenylporphyrin $\text{Ru}(\text{O})_2(\text{TPP})$ over a period of 96 h [44]. Instead (*Z*)-isomers, compounds with a molecular weight of m/z 553 = $[M_{\text{lycopene}} + 16 + \text{H}]^+$, which we called lycopene monoxides, and cleavage compounds assigned to apo-lycopenals [44]. Almost all the apo-lycopenals/ones that were obtained by oxidation of lycopene with potassium permanganate were detected, except the short-chain apo-5-lycopenone and apo-7-lycopenal (44). Thus, our results may suggest that HOCl behaves as a stronger oxidant with a higher oxidation potential than permanganate and other oxidants for lycopene destruction.

Many of the metabolites in groups 1 and 2 have been reported as their metabolic pathways were investigated extensively while other cleavage products and their functions are either

not yet fully explained or not mentioned before in the literature. For example, earlier *in vitro* studies by Ben-Aziz *et al.* have shown that apo-6'- and apo-8'- lycopene were obtained by oxidation of lycopene with potassium permanganate [42]. Cross-sequential studies by Ukai *et al.* described a protocol for the reaction which gave apo-6'-lycopenal as the main product after 44 hours, whereas 50% of lycopene remained intact [43]. Caris-Veyrat *et al.* have latter optimized the experimental conditions by modifying the solvent mixture and the phase transfer catalyst to complete oxidized lycopene and obtained mono oxidative cleavage compounds as major products [44]. After the complete disappearance of lycopene, they detected eight apo-lycopenals giving a range of products from the longest apo-6'-lycopenal to the shortest one detected apo-5-lycopenone, three apo-lycopenones, and six apo-carotendials [45]. Using HPLC analysis with UV-visible detection Kim *et al.* have tentatively identified mono cleavage compounds of lycopene [46]. Exposure of lycopene to atmospheric oxygen and perfusion of ozone has led to tentatively identify a (E, E, E)-4-methyl-8-oxo-2,4,6-nonatrienal [45]. Kim *et al.* isolated and identified a cleavage product of lycopene from an autoxidation mixture of lycopene products [46].

Previous studies have also shown that lycopene undergoes oxidative degradation *in vivo* [46]. Some of the lycopene cleavage products identified in this work (Table 1) have been shown to participate in biological effects in animal models and cell culture system. Indeed, several of lycopene metabolites have been found in human milk and serum [47]. Consistent with our current finding, these studies have concluded that lycopene can unselectively been cleaved at any double bond position in nonenzymatic manner when organisms are subjected to oxidative stress (33,44,45,48). Since several of the HOCl-mediated lycopene cleavage products of different chain lengths assigned in group 1 and 2 (Table I) have been identified and shown to be associated with reduced risk of certain cancers [49–52], we believe that our work could be biologically relevant. Indeed, it has been thought that lycopene metabolites may induce changes in the gene level attaining the expression of relevant genes and serve as anticancer agents [53]. Related studies by Nara *et al.* have shown that a mixture of oxidation products of lycopene induced apoptosis in HL-60 cells following incubation for 24 h, at 37°C [54]. Kotake-Nara *et al.* have shown that acyclo-retinoic acid, the centrally cleaved metabolite of lycopene, reduces cell viability by inducing apoptosis in human prostate cancer cells [55]. Aust *et al.*, have show that 2,7,11-trimethyl-tetradecaheptaene-1,14-dial, generated by lycopene degradation, products in cell signaling enhancing cell-to-cell communication through gap junctions in rat liver epithelial WB-F344 cells [56]. In addition, apo-10'-lycopenoic acid was also found to be a lycopene metabolite in ferret lung tissue [57]. In parallel, several other lycopene metabolites with different functional groups have previously been reported, for example, 5,6-dihydroxy-5',6'-dihydrolycopene and 2,6-cyclolycopene-1,5-diol A and B have been found in the extracts of human serum/plasma [47,58].

Identifying similarities and differences in the interactions between HOCl and various oxidants yields valuable mechanistic insights into the potential biochemical and functional significance of HOCl-lycopene interactions both *in vitro* and *in vivo*. On the basis of the present results, previous published studies of the interaction of HOCl with carotenoids, and the exposure of lycopene to different oxidants (e.g., permanganates and metalloporphyrins), we have generated a stepwise scheme that shows possible pathways for the generation of different lycopene cleavage products after the exposure to increasing concentration of HOCl (Fig. 7). In this reaction, lycopene interacts with the HOCl molecule in which the chloride atom of HOCl acts as an electrophile and the electron rich olefin initially acts as the nucleophile. When the Cl atom adds across the double bond, it does so in such a mannar in which a pseudo-secondary carbo-cation is formed transforming to a more stable chloronium ion. Addition of the hydroxide proceeds by obeying the regioselectivity of nucleophilic chloronium ion ring opening. The semi-halohydrin then undergoes an epoxide ring forming

reaction via an intramolecular S_N2 type in which a chlorine atom is displaced. Reaction of the epoxide with a second molecule of HOCl (deprotonated by Cl^-) causes cleavage of the terminal carbon-carbon bond and the generation of an aldehyde (which is one carbon shorter than the parent epoxide from which it was formed). Further oxidation of the aldehyde by a third molecule of HOCl leads to the formation of carboxylic acid. Thus, it is evident that one molecule of lycopene (which has thirteen $C=C$) has the potential capacity to scavenge multiple molecules of HOCl.

The degree of reproducibility and similarity of the isolates increased progressively when the LC/MS analyses were carried out immediately after the extraction of the cleavage products in chloroformic solutions and subsequently as dry powders. The delay of 20–45 h in LC/MS analysis caused a noticeable decrease in the chlorinated compounds (group 3), indicating that the chlorinated molecules are relatively unstable and may decay to non-chlorinated species such as aldehydes and carboxylic acids. The metabolites in groups 3 and their metabolic pathways have not been previously reported. The excessive HOCl generated under inflammatory conditions when phagocytes are activated and MPO is released may play a harmful role due to its ability to react eagerly with a variety of biological molecules, and its ability to chlorinate as well as oxidize biomolecules [59]. Indeed, there is substantial evidence that MPO and HOCl play a role in atherosclerosis, diabetes and asthma, and HOCl-mediated tissue injury has also been found to result in an increase in inflammatory disorders as determined by increased levels of free and protein-bound chlorotyrosine and α -chloro fatty aldehydes [reviewed in 60 and 61].

The generation of a series of chlorohydrins of cholesterol[6- β -chloro-cholestane-(3 β ,5 α)-diol, 5- α -chloro-cholestane-(3 β ,6 β)-diol and 6- α -chloro-cholestane-(3 β ,5 β)-diol has been observed following exposure to HOCl, as well as dichlorinated product, 5,6-dichloro-cholestane-3 β -ol [62,63]. In addition to electrophilic addition, HOCl can utilize N-halogenation reactions at amines in the head groups of phosphatidylethanolamines and phosphatidylserines, yielding corresponding chloramines [64,65]. These intermediate products are initial short lived molecules, and tend to decay to a more stable non-chlorinated species such as aldehydes. HOCl reacts very rapidly with the sulfur-containing side chains of methionine and cysteine residues and, at a slightly lower rate constant, with amines and other nitrogen-containing residue [66, 67]. The ability of lycopene to scavenge HOCl formed during inflammatory situations is more likely to prevent proteins and unsaturated phospholipids from MPO- and HOCl-induced damage. Thus reduced lycopene availability and/or increased consumption as assessed by the degree of its fragmentation and modification may reflect the basic mechanism of increasing the risk of diseases induced by MPO and HOCl.

In summary, inhibition of MPO or removing its downstream final product, HOCl, is an attractive target for preventing HOCl-mediated tissue injury and progression of inflammatory diseases. In this study we advance the current knowledge of lycopene antioxidant properties and show for the first time that lycopene may serve as a potent scavenger of HOCl. The interplay between lycopene and HOCl may have a broad implication in the function of inflammatory biological systems throughout the body. Further evaluation is necessary to assess whether lycopene could represent an interventional approach to minimize the deleterious effects associated with inflammation.

The abbreviations used are:

ARE	antioxidant response element
Cl^-	chloride

DNA	deoxyribonucleic acid
FIA	flow injection analysis
H₂O₂	hydrogen peroxide
HOCl	hypochlorous acid
HPLC	High Performance Liquid Chromatography
LC/APCI/MS	Liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry
<i>m/z</i>	mass to charge ratio
MPO	Myeloperoxidase
RNA	ribonucleic acid
ROS	reactive oxidant species
DNA	deoxyribonucleic acid

References

1. Agarwal S, Rao AV. Tomato lycopene and its role in human health and chronic diseases. *Can. Med. Assoc. J.* 2000; 163:739–744. [PubMed: 11022591]
2. Khachik F, Carvalho L, Bernstein PS, Muir GJ, Zhao DY, Katz NB. Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. *Exp. Biol. Med.* 2002; 227:845–851.
3. Matos HR, Di Mascio P, Medeiros MH. Protective effect of lycopene on lipid peroxidation and oxidative DNA damage in cell culture. *Arch Biochem Biophys.* 2000; 383:56–59. [PubMed: 11097176]
4. Voutilainen S, Nurmi T, Mursu J, Rissanen TH. Carotenoids and cardiovascular health. *Am J Clin Nutr.* 2006; 83:1265–1271. [PubMed: 16762935]
5. Dahan K, Fennel M, Kumar NB. Lycopene in the prevention of prostate cancer. *J Soc Integr Oncol.* 2008; 6:29–36. [PubMed: 18302908]
6. Rao AV. Lycopene, tomatoes, and the prevention of coronary heart disease. *Exp Biol Med* (Maywood). 2002; 227:908–913. [PubMed: 12424333]
7. Heber D. Colorful cancer prevention: alpha-carotene, lycopene, and lung cancer. *Am J Clin Nutr.* 2000; 72:901–902. [PubMed: 11010928]
8. Zhao YP, Yu WL, Hu WL, Ying Y. Anti-inflammatory and anticoagulant activities of lycopene in mice. *Nutrition Research.* 2003; 23:1591–1595.
9. Saedisomeolia A, Wood LG, Garg ML, Gibson PG, Wark PA. Lycopene enrichment of cultured airway epithelial cells decreases the inflammation induced by rhinovirus infection and lipopolysaccharide. *J Nutr Biochem.* 2009; 20:577–585. [PubMed: 18824341]
10. Subhash K, Bose C, Agrawal BK. Effect of short term supplementation of tomatoes on antioxidant enzymes and lipid peroxidation type-II diabetes. *Indian Journal of Clinical Biochemistry.* 2007; 22:95–98. [PubMed: 23105660]
11. Tang X, Yang X, Peng Y, Lin J. Protective effects of lycopene against H₂O₂-induced oxidative injury and apoptosis in human endothelial cells. *Cardiovasc Drugs Ther.* 2009 (ahead of print).
12. Mein JR, Lian F, Wang X-D. Biological activity of lycopene metabolites: implications for cancer prevention. *Nutrition Reviews.* 2008; 66:667–683. [PubMed: 19019036]
13. Zhang LX, Cooney RV, Bertram JS. Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemopreventive action. *Carcinogenesis.* 1991; 12:2109–2114. [PubMed: 1934296]

14. Klebanoff, SJ. Oxygen Metabolites from Phagocytes. In: Gallin, JI.; Snyderman, R., editors. *Inflammation: Basic Principles and Clinical Correlates*. Philadelphia: Lippincott Williams & Wilkins; 1999. p. 721-768.
15. Pullar JM, Vissers MC, Winterbourn CC. Living with a killer: the effects of hypochlorous acid on mammalian cells. *IUBMB Life*. 2000; 50:259–266. [PubMed: 11327319]
16. Malech HL, Gallin JI. Neutrophils in human diseases. *N. Engl. J. Med*. 1987; 317:687–694. [PubMed: 3041216]
17. Malle E, Buch T, Grone HJ. Myeloperoxidase in kidney disease. *Kidney Int*. 2003; 64:956–967.
18. Ohshima H, Tatemichi M, Sawa T. Chemical basis of inflammation-induced carcinogenesis. *Arch Biochem Biophys*. 2003; 417:3–11. [PubMed: 12921773]
19. Schiller J, Fuchs B, Arnhold J, Arnold K. Contribution of reactive oxygen species to cartilage degradation in rheumatic diseases: molecular pathways, diagnosis and potential therapeutic strategies. *Curr Med Chem*. 2003; 20:2123–2145. [PubMed: 12871089]
20. Hazen SL, Heinecke JW. 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. *J. Clin. Invest*. 1997; 99:2075–2081. [PubMed: 9151778]
21. Goud AP, Goud PT, Diamond MP, Gonik B, Abu-Soud HM. Reactive oxygen species and oocyte aging: role of superoxide, hydrogen peroxide, and hypochlorous acid. *Free Radic Biol Med*. 2008; 44:1295–1304. [PubMed: 18177745]
22. Prutz WA. Hypochlorous acid interactions with thiols, nucleotides, DNA, and other biological substrates. *Arch Biochem Biophys*. 1996; 332:110–120. [PubMed: 8806715]
23. Prutz WA. Interactions of hypochlorous acid with pyrimidine nucleotides, and secondary reactions of chlorinated pyrimidines with GSH, NADH, and other substrates. *Arch Biochem Biophys*. 1998; 349:183–191. [PubMed: 9439597]
24. Bergt C, Pennathur S, Fu X, Byun J, O'Brien K, McDonald TO, Singh P, Anantharamaiah GM, Chait A, Brunzell J, Geary RL, Oram JF, Heinecke JW. The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. *Proc Natl Acad Sci U S A*. 2004; 101(35):13032–13037. [PubMed: 15326314]
25. Winterbourn CC, van den Berg JJ, Roitman E, Kuypers FA. Chlorohydrin formation from unsaturated fatty acids reacted with hypochlorous acid. *Arch. Biochem. Biophys*. 1992; 296:547–555. [PubMed: 1321589]
26. Zhang R, Brennan ML, Shen Z, MacPherson JC, Schmitt D, Molenda CE, Hazen SL. Myeloperoxidase functions as a major enzymatic catalyst for initiation of lipid peroxidation at sites of inflammation. *J Biol Chem*. 2002; 277:46116–46122. [PubMed: 12359714]
27. Candeias LP, Patel KB, Stratford MRL, Wardman P. Free hydroxyl radicals are formed on reaction between the neutrophil-derived species superoxide anion and hypochlorous acid. *FEBS Lett*. 1993; 333:151–153. [PubMed: 8224156]
28. Long CA, Bielski BHJ. Rate of reaction of superoxide radical with chloride-containing species. *J. Phys. Chem*. 1980; 84:555–557.
29. Held AM, Halko DJ, Hurst JK. Mechanisms of chlorine oxidation of hydrogen peroxide. *J. Am. Chem. Soc*. 1978; 100:5732–5740.
30. Galijasevic S, Abdulhamid I, Abu-Soud HM. Melatonin is a potent inhibitor for myeloperoxidase. *Biochemistry*. 2008; 47:2668–2677. [PubMed: 18237195]
31. Galijasevic S, Abdulhamid I, Abu-Soud HM. Potential role of tryptophan and chloride in the inhibition of human myeloperoxidase. *Free Radic Biol Med*. 2008; 44:1570–1577. [PubMed: 18279680]
32. Sliskovic I, Abdulhamid I, Sharma M, Abu-Soud HM. Analysis of the mechanism by which tryptophan analogs inhibit human myeloperoxidase. *Free Radic Biol Med*. 2009; 47:1005–1013. [PubMed: 19596067]
33. dos Anjos Ferreira AL, Yeum KJ, Russell RM, Krinsky NI, Tang G. Enzymatic and oxidative metabolites of lycopene. *J Nutr Biochem*. 2004; 15:493–502. [PubMed: 15378815]

34. Sutherland WH, de Jong SA, Walker RJ. Hypochlorous acid and low serum paraoxonase activity in haemodialysis patients: an in vitro study. *Nephrol Dial Transplant*. 2004; 19:75–82. [PubMed: 14671042]
35. Kettle AJ, Winterbourn CC. Assays for the chlorination activity of myeloperoxidase. *Methods Enzymol*. 1994; 233:502–512. [PubMed: 8015486]
36. Sommerburg O, Langhans CD, Arnhold J, Leichsenring M, Salerno C, Crifò C, Hoffmann GF, Debatin KM, Siems WG. Beta-carotene cleavage products after oxidation mediated by hypochlorous acid--a model for neutrophil-derived degradation. *Free Radic Biol Med*. 2003; 35:1480–1490. [PubMed: 14642396]
37. Pattison DI, Hawkins CL, Davies MJ. What are the plasma targets of the oxidant hypochlorous acid? A kinetic modeling approach. *Chem Res Toxicol*. 2009; 22:807–817. [PubMed: 19326902]
38. Schaffer SW, Azuma J, Mozaffari M. Role of antioxidant activity of taurine in diabetes. *Can J Physiol Pharmacol*. 2009; 87:91–99. [PubMed: 19234572]
39. Bouckennooghe T, Remacle C, Reusens B. Is taurine a functional nutrient? *Curr Opin Clin Metab Care*. 2006; 9:728–733.
40. Giovannucci E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *J Natl Cancer Inst*. 1999; 91:317–331.
41. Arab L, Steck S. Lycopene and cardiovascular disease. *Am J Clin Nutr*. 2000; 71:1691S–1695S. [PubMed: 10837319]
42. Ben-Aziz A, Britton G, Goodwin TW. Carotene Epoxides of *Lycopersicon esculentum*. *Phytochemistry*. 1973; 12:2759–2764.
43. Ukai N, Lu Y, Etoh H, Tagi A, Ina K, Oshima S, Ojima F, Sakamoto H, Ishiguro Y. Photosensitized oxygenation of lycopene. *Biosci. Biotech. Biochem*. 1994; 58:1718–1719.
44. Caris-Veyrat C, Schmid A, Carail M, Bohm V. Cleavage products of lycopene produced by in vitro oxidations: characterization and mechanisms of formation. *J Agric Food Chem*. 2003; 51:7318–7325. [PubMed: 14640578]
45. Zhang H, Kotake-Nara E, Ono H, Nagao A. A novel cleavage product formed by autoxidation of lycopene induces apoptosis in HL-60 cells. *Free Radic Biol Med*. 2003; 35:1653–1663. [PubMed: 14680688]
46. Kim S-J, Nara E, Kobayashi H, Terao J, Nagao A. Formation of cleavage products by autoxidation of Lycopene. *Lipids*. 2001; 36:191–199. [PubMed: 11269700]
47. Khachik F, Spangler CJ, Smith JC Jr, Canfield LM, Steck A, Plander H. Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. *Anal Chem*. 1997; 69:1873–1881. [PubMed: 9164160]
48. Gajic M, Zaripheh S, Sun F, Erdman JW Jr. Apo-8'-Lycopene and Apo-12'-Lycopene Are Metabolic Products of Lycopene in Rat Liver. *J Nutr*. 2006; 136:1552–1557. [PubMed: 16702320]
49. Hu X, White KM, Jacobsen NE, Mangelsdorf DJ, Canfield LM. Inhibition of Growth and Cholesterol Synthesis in Breast Cancer Cells by Oxidation Products of β -Carotene. *J. Nutr. Biochem*. 1998; 9:567–574.
50. Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. Intake of Carotenoids and Retinol in Relation to Risk of Prostate Cancer. *J. Natl. Cancer Inst*. 1995; 87:1767–1776. [PubMed: 7473833]
51. Gann PH, Ma J, Giovannucci E, Willett W, Sacks FM, Hennekens CH, Stampfer MJ. Lower Prostate Cancer Risk in Men with Elevated Plasma Lycopene Levels: Results of a Prospective Analysis. *Cancer Res*. 1999; 59:1225–1230. [PubMed: 10096552]
52. Chen G, Djuric Z. Detection of 2,6-cyclolycopene-1,5-diol in breast nipple aspirate fluids and plasma: a potential marker of oxidative stress. *Cancer Epidemiol Biomarkers Prev*. 2002; 11:1592–1596. [PubMed: 12496049]
53. Freemantle SJ, Spinella MJ, Dmitrovsky E. Retinoids in cancer therapy and chemoprevention: promise meets resistance. *Oncogene*. 2003; 22:7305–7315. [PubMed: 14576840]
54. Nara E, Hayashi H, Kotake M, Miyashita K, Nagao A. Acyclic carotenoids and their oxidation mixtures inhibit the growth of HL-60 human promyelocytic leukemia cells. *Nutr Cancer*. 2001; 39:273–283. [PubMed: 11759292]

55. Kotake-Nara E, Kim SJ, Kobori M, Miyashita K, Nagao A. Acyclo-retinoic acid induces apoptosis in human prostate cancer cells. *Anticancer Res.* 2002; 22:689–695. [PubMed: 12014638]
56. Aust O, Ale-Agha N, Zhang L, Wollersen H, Sies H, Stahl W. Lycopene oxidation product enhances gap junctional communication. *Food Chem. Toxicol.* 2003; 41:1399–1407. [PubMed: 12909274]
57. Lian F, Smith DE, Ernst H, Russell RM, Wang X-D. Apo-10'-lycopenoic acid inhibits lung cancer cell growth in vitro, and suppresses lung tumorigenesis in the A/J mouse model in vivo. *Carcinogenesis.* 2007; 28:1567–1574. [PubMed: 17420169]
58. Khachik F, Beecher GR, Smith JC Jr. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. *J Cell Biochem Suppl.* 1995; 22:236–246. [PubMed: 8538204]
59. Henderson JP, Byun J, Heinecke JW. Molecular chlorine generated by the myeloperoxidase-hydrogen peroxide-chloride system of phagocytes produces 5-chlorocytosine in bacterial RNA. *J Biol Chem.* 1999; 274:33440–33448. [PubMed: 10559226]
60. Nicholls SJ, Hazen SL. Myeloperoxidase, modified lipoproteins, and atherogenesis. *J Lipid Res.* 2009; 50(Suppl):S346–S351. [PubMed: 19091698]
61. Vivekanandan-Giri A, Wang JH, Byun J, Pennathur S. Mass spectrometric quantification of amino acid oxidation products identifies oxidative mechanisms of diabetic end-organ damage. *Reviews in Endocrine and Metabolic Disorders.* 2008; 9:275–287. [PubMed: 18752069]
62. Hazen SL, Hsu FF, Duffin K, Heinecke JW. Molecular chlorine generated by the myeloperoxidase-hydrogen peroxide-chloride system of phagocytes converts low density lipoprotein cholesterol into a family of chlorinated sterols. *J. Biol. Chem.* 1996; 271:23080–23088. [PubMed: 8798498]
63. Carr AC, van den Berg JJ, Winterbourn CC. Chlorination of cholesterol in cell membranes by hypochlorous acid. *Arch. Biochem. Biophys.* 1996; 332:63–69. [PubMed: 8806710]
64. Pattison DI, Hawkins CL, Davies MJ. Hypochlorous acid-mediated oxidation of lipid components and antioxidants present in low-density lipoproteins: absolute rate constants, product analysis, and computational modeling. *Chem. Res. Toxicol.* 2003; 16:439–449. [PubMed: 12703960]
65. Kawai Y, Kiyokawa H, Kimura Y, Kato Y, Tsuchiya K, Terao J. Hypochlorous acid-derived modification of phospholipids: characterization of aminophospholipids as regulatory molecules for lipid peroxidation. *Biochemistry.* 2006; 45:14201–14211. [PubMed: 17115715]
66. Pattison DI, Davies MJ. Reactions of myeloperoxidase derived oxidants with biological substrates: gaining chemical insight into human inflammatory diseases. *Curr. Med. Chem.* 2006; 13:3271–3290. [PubMed: 17168851]
67. Pitt AR, Spickett CM. Mass spectrometric analysis of HOCl- and free-radical-induced damage to lipids and proteins. *Biochem Soc Trans.* 2008; 36:1077–1082. [PubMed: 18793192]

Figure 1.
Structure of all *trans*-lycopene. The number represents the carbon number in hydrocarbon chain.

Figure 2.

Lycopene spectral changes as a function of HOCl concentration. Absorbance spectra of lycopene alone (top most spectra) and after reaction with 50, 125, 250, 500, 750, 1250, 1875, 2500 and 3750 μM HOCl, respectively (from top to bottom). The arrows show the direction of the absorbance decrease as a function of increasing HOCl ratio. The inset shows the color change of lycopene solution (50 μM) when reacted with 3750 μM of HOCl.

A

B

Figure 3.

A: HPLC analysis of the HOCl induced lycopene oxidation products. The figure shows the chromatograms obtained at 450 nm, when lycopene (50 μM) was reacted with different concentrations of HOCl. Panel A: 0 μM, B: 250 μM, C: 500 μM and D: 625 μM HOCl respectively. The chromatograms are the representative set from three independent experiments.

B: Percent of lycopene remaining after reaction with different concentrations of HOCl. 50 μM of lycopene was reacted with different concentrations of HOCl (0, 250, 500, 625 μM respectively) and after HPLC analysis (for details see *Materials and Methods* section) the area under the curve for lycopene signal was integrated. The above result is the mean of three independent experiments and the error bars represent the Standard Error of Measurement.

Figure 4. Liquid Chromatography atmospheric pressure chemical ionization mass spectrometry (positive mode) of reaction of lycopene with HOCl (lycopene:oxidant ratio 1:5). The reaction mixture was separated by reverse phase HPLC and subjected to APCI/MS as described in *Materials and Methods*. The major reaction product produced an intense peak at 2.86 min. Examination of the MS spectrum revealed that the $[M + H]^+$ ion had a m/z 393. The extracted ion chromatogram (Panel A) and the MS spectrum of this peak (Panel B) are depicted.

Figure 5. Liquid Chromatography atmospheric pressure chemical ionization mass spectrometry (positive mode) of reaction of lycopene with HOCl (lycopene:oxidant ratio 1:5). The reaction mixture was separated by reverse phase HPLC and subjected to APCI/MS as described in Methods. The second major reaction product produced an intense peak at 1.75 min. Examination of the MS spectrum revealed that the $[M + H]^+$ ion had a m/z 349. The extracted ion chromatogram (Panel A) and the MS spectrum of this peak (Panel B) are depicted.

Figure 6.

Novel reaction products of lycopene with HOCl (lycopene:oxidant ratio 1:20). The reaction mixture was separated by reverse phase HPLC and subjected to APCI/MS as described in Methods. Two new metabolites were detected at retention times 1.86 min and 2.14 min. Examination of the MS spectrum revealed that the $[M + H]^+$ ion had a m/z 443. The extracted ion chromatogram is shown in Panel A. The MS spectrum of the peak at 1.86 min (Panel B) and 2.14 min (Panel C) are depicted. Note presence of one chlorine atom as the ion intensity of $[M + H + 2]^+$ ion is approximately 40% of $[M + H]^+$ indicating chlorine isotope pattern in Panel B. In contrast, the isotopic pattern of m/z 443 from the second peak has no intense $[M + H + 2]^+$ ion (Panel C) indicating that this product is not chlorinated.

Figure 7.
Proposed chemical mechanism showing the stepwise oxidation of lycopene by HOCl.

Table 1

Possible structures and names of the metabolites and oxidation fragments as tentatively identified from the m/z values. The term 'ratio' in the table denotes the molar ratio between lycopene:HOCl, where that particular fragment was observed.

Ratio	m/z	Proposed Structure	Formula	Mol. wt.	Name
1:5, 1:10, 1:12.5, 1:15, 1:20	301		$C_{20}H_{28}O_2$	300.44	Acycloretonic acid
1:15, 1:20	311		$C_{20}H_{24}O_3$	312.4	Crocin Semialdehyde
1:10, 1:12.5, 1:15, 1:20	325		$C_{22}H_{30}O_2$	326.47	Apo-14' -lycopenoic acid
1:5, 1:15	329		$C_{20}H_{24}O_4$	328.4	Crocin
1:10, 1:12.5, 1:15	339		$C_{22}H_{28}O_3$	340.46	18 formyl 2,4,6,8,10,12,14 octadecaheptenoic acid 2,7,11,14 tetra methyl
1:5, 1:15	349		$C_{24}H_{28}O_2$	348.48	Apo-5,6' - carotendial
1:5	391		$C_{27}H_{34}O_2$	390.56	Apo-1,6' - carotendial

Ratio	m/z	Proposed Structure	Formula	Mol. wt.	Name
1:5, 1:10, 1:15, 1:20	393		$C_{27}H_{36}O_2$	392.57	Apo-10' -lycopenoic acid
1:15	421		$C_{27}H_{34}O_4$	422.56	4, 6, 8, 10, 12, 14, 16, 18, 20 Docosonene 1, 22 dioic acid 4, 8, 12, 17, 21 penta methyl
1:5	431		$C_{29}H_{36}O_3$	432.59	24 formyl 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 Tetracosonene 4, 8, 13, 17, 20 penta methyl
1:20	443		$C_{32}H_{42}O$	442.68	Apo-6' -lycopenal
1:20	443		$C_{27}H_{35}ClO_3$	443.02	2 hydroxy 3 chloro 4, 6, 8, 10, 12, 14, 16, 18 Docosonene 1, 22 dial 6, 11, 15, 19 tetra methyl
1:20	449		$C_{29}H_{36}O_4$	448.59	2, 4, 6, 8, 10, 12, 14, 16, 18, 20 Tetracosonene 1, 24 oic acid 4, 8, 13, 17, 21 penta methyl