Quantification of apo-lycopenals in food products and human blood plasma

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Abstract:

Consumption of tomato and tomato products is correlated with a reduced incidence of a number of diseases. Research has suggested that lycopene, the most predominant carotenoid in tomatoes, may be responsible for this effect. However, researchers have also suggested that lycopene may be metabolized in mammals by eccentric cleavage to biologically active aldehydes, in an analogous fashion to cleavage of ß-carotene, catalyzed by ß-carotene oxygenase. Since apo-6'- and apo-8'-lycopenal have been reported earlier in raw tomato, we hypothesized that several other apo-lycopenals may be present in raw and processed foods, as well as blood plasma. Apo-lycopenal standards were prepared by in vitro oxidation of lycopene, and an HPLC-MSMS method using atmospheric pressure chemical ionization in negative mode was developed to separate and detect the apo-6'-, 8'-, 10'-, 12'-, 14'-, and 15'-lycopenal products formed in the reaction. Hexane/acetone extracts of raw tomato, grapefruit, watermelon, and processed tomato products were analyzed, as well as blood plasma of individuals who had consumed tomato juice for 8 weeks. Apo-6'-, 8'-, 10'-, 12'-, and 14'-lycopenals were detected and quantified in all food products tested, as well as blood plasma. The sum of apo-lycopenals was 6.5µg/100g roma tomato, 73.4 µg/100g tomato paste, and 1.88nmol/L of blood plasma. We conclude that several apo-lycopenals, in addition to apo-6'- and 8'-, are present in lycopene containing foods. In addition, the presence of apo-lycopenals in plasma may partly derive from the absorption of apo-lycopenals directly from food.

Introduction:

Consumption of tomatoes and tomato products has been associated with a reduction in risk for a number cancers as well as heart disease[1] . Tomato products contain a high level of the carotenoid lycopene, and researchers have hypothesized that lycopene or a metabolite of lycopene may be involved in modifying these disease processes. However, the metabolism of lycopene is poorly understood.

Oxidative breakdown products of lycopene have been recently observed in animal models. Apo-10'-lycopenol was found in lung tissue of smoking ferrets consuming lycopene[2]. In addition, apo-8'-lycopenal and a compound presumed to be apo-12'-lycopenal have been reported in rats fed a lycopene-containing diet[3]. Investigators have speculated that the enzyme beta-carotene oxygenase 2 (BCO2) is responsible for the formation of these oxidative products of lycopene.

A search of the literature reveals that apo-6'-lycopenal and apo-8'-lycopenal have been previously identified in raw tomatoes and tomato paste[4,5].

Our objectives were to determine whether:

1.Additional apo-lycopenals are present in tomatoes.
2.Apo-lycopenals are present in other lycopene containing foods.
3.Apo-lycopenals are present in the blood plasma of humans consuming a diet high in tomato products.
4. Quantify the levels of these products in foods and blood plasma

Materials and Methods:

- Apo-lycopenals were synthesized using an ozonolysis method
- Compounds were separated using reverse phase HPLC method and identified on a quadrupole time-of-flight hybrid mass spectrometer interfaced with the HPLC via an atmospheric pressure chemical ionization (APcI) source operated in negative ion mode
- Foods were extracted using a well established hexane/acetone (1:1) method[6]. Raw fruits were pureed in a blender before extraction.
- Three brands of each tomato product was tested. Extractions were performed in duplicate.
- Human blood plasma was extracted using hexane/ethanol/acetone/toluene (HEAT) mixture[7]
- Apo-6'-, apo-8', and apo-12'-lycopenal standards used for quantitation were purchased. Lycopene was isolated from tomato paste and crystallized using an in-house method

Materials and Methods, cont.:

- Quantitation of apo-lycopenals in food and plasma samples was performed on a triple quadrupole mass spectrometer interfaced with a UPLC via an APcI source operated in negative ion mode.

Results:

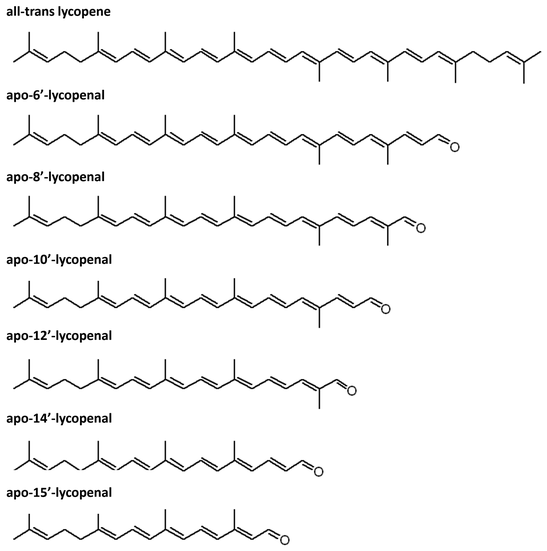

Figure 1. Chemical structures of all-trans lycopene and long chain apo-lycopenals observed after oxidation of lycopene with ozone.

- Identity of apo-6', apo-8', and apo-12'-lycopenal were confirmed by retention time (RT) coincident with commercial standards, UV spectra, and loss of the terminal isoprenyl group (M-69) after fragmentation by the mass spectrometer (MS/MS)
- Apo-10' and apo-14'-lycopenal were identified with putative apo-10' and 14' observed in oxidation mixtures corresponding to anticipated RT and characteristic M-69 fragmentation.

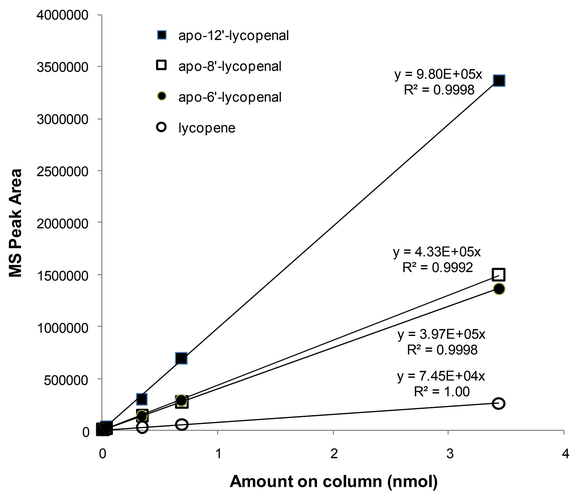

Figure 2. Standard curves of apo-12'-lycopenal, apo-8'-lycopenal, apo-6'-lycopenal, and lycopene.

Table 1. Apo-lycopenals in commonly consumed lycopene containing foods

Food	µg/100g wet weight lycopene	µg/100g wet weight apo-6'-lycopenal	apo-8'-lycopenal	apo-10'-lycopenal[a]	apo-12'-lycopenal	apo-14'-lycopenal[b]
Roma Tomato	3,452	1.1	1.8	1.1	2.2	0.3
Grape Tomato	6,166	1.6	2.2	1.2	2.3	0.4
Red Vine Ripened Tomato	3,778	3.5	4.3	2.0	2.9	0.4
Ruby Red Grapefruit	187	0.02	0.03	0.02	0.05	nd
Watermelon	3,154	0.8	0.9	0.5	0.8	0.2
Catsup	8,721	4.9	9.3	1.2	5.5	0.3
Spaghetti Sauce	11,192	6.3	10	1.2	5.3	0.3
Pizza Sauce	11,846	10	16	1.7	7.1	0.4
Tomato Soup	14,131	6.9	11	1.7	6.1	0.5
Tomato Paste	8,733	19	34	3.7	16	0.7
Tomato Juice	11,392	1.8	5.0	0.6	2.8	0.1

nd = not detected
[a]Level was estimated by averaging apo-8'-lycopenal and apo-12'-lycopenal slopes.
[b]Level was estimated by using apo-12'-lycopenal equivalents.

- Levels reported are the average of duplicates for raw fruits and vegetables, and the average across three brand name products for processed tomatoes.
- Apo-lycopenals (and lycopene) were highest in tomato paste
- The range of values for a particular apo-lycopenal species was 50% to 200% across different brands of a given product.

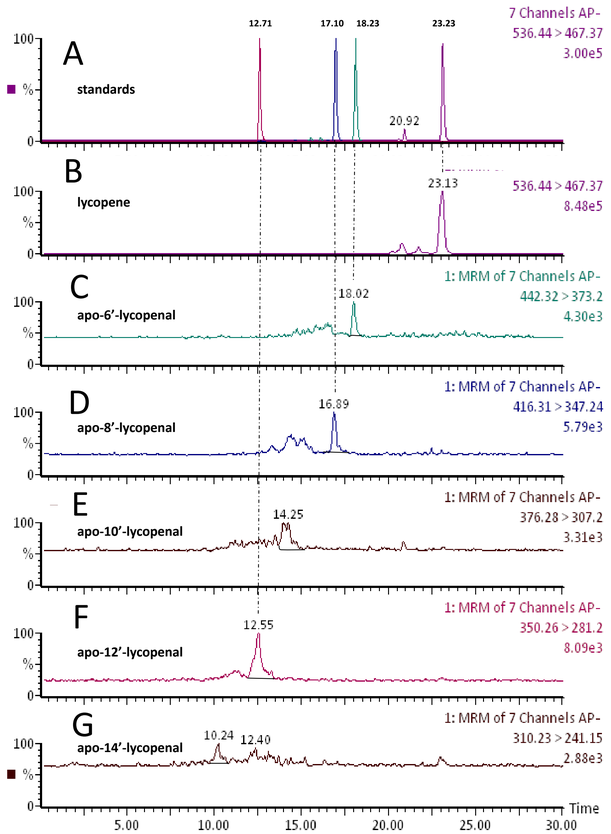

Figure 3. Mass chromatograms showing lycopene and apo-lycopenal standards (A) vs. blood plasma extract (B-G) of a human subject. (A) Overlay of MRM traces corresponding to apo-12', 8', 6'-lycopenal and lycopene standards. Lycopenoids in blood plasma: (B) lycopene, (C) apo-6'-lycopenal, (D) apo-8'-lycopenal, (E) apo-10'-lycopenal, (F) apo-12'-lycopenal, (G) apo-14'-lycopenal.

Table 2. Apo-lycopenals in blood plasma of subjects consuming a high tomato juice diet for 8 weeks.

	nmol/L lycopene	nmol/L apo-6'-lycopenal	apo-8'-lycopenal	apo-10'-lycopenal[a]	apo-12'-lycopenal	apo-14'-lycopenal[b]
Average	1,089	0.12	0.63	0.28	0.73	0.12
(Range)	(700-1,833)	(0.06-0.23)	(0.29-1.17)	(0.14-0.45)	(0.23-1.13)	(0.08-0.18)

[a]Level was estimated by averaging apo-8'-lycopenal and apo-12'-lycopenal slopes.
[b]Level was estimated by using apo-12'-lycopenal equivalents.

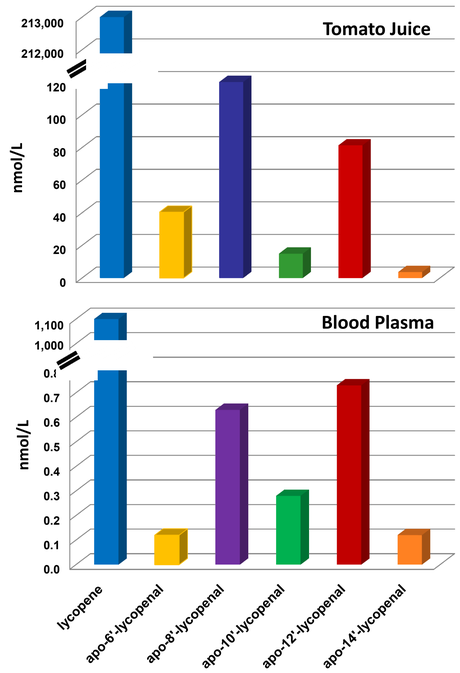

Figure 4. A comparison of the profile of lycopenoids in tomato juice and blood plasma of individuals consuming tomato juice. Notice similarity.

Conclusions:

- To the best of our knowledge, this is the first report of apo-lycopenals observed in humans
- An entire series of monoaldehyde lycopenals (apo-6',8', 10', 12', 14') was observed in all foods tested. Apo-15'-lycopenal was not observed in food products or blood plasma tested
- The level of apo-lycopenals in food products and blood plasma is roughly 1,000 times lower than the level of lycopene, but on par with levels of known carotenoid metabolites such as retinoic acid
- The superior resolving power, sensitivity, and selectivity of HPLC-MS/MS, in combination with known standards, is essential for the detection and quantification of these compounds
- This research suggests that apo-lycopenals observed in blood plasma may be at least partly derived from the diet

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