A Review of Animal Model Studies of Tomato Carotenoids, Lycopene, and Cancer Chemoprevention

LEONARD A. COHEN[1]
American Health Foundation, Valhalla, New York 10595

There are relatively few reports on the cancer chemopreventive effects of lycopene or tomato carotenoids in animal models. The majority, but not all, of these studies indicate a protective effect. Inhibitory effects were reported in two studies using aberrant crypt foci, an intermediate lesion leading to colon cancer, as an end point and in two mammary tumor studies, one using the dimethylbenz(a)anthracene model, and the other the spontaneous mouse model. Inhibitory effects were also reported in mouse lung and rat hepatocarcinoma and bladder cancer models. However, a report from the author's laboratory found no effect in the *N*-nitrosomethylurea-induced mammary tumor model when crystalline lycopene or a lycopene-rich tomato carotenoid oleoresin was administered in the diet. Unfortunately, because of differences in routes of administration (gavage, intraperitoneal injection, intra-rectal instillation, drinking water, and diet supplementation), species and strain differences, form of lycopene (pure crystalline, beadlet, mixed carotenoid suspension), varying diets (grain-based, casein based) and dose ranges (0.5–500 ppm), no two studies are comparable. It is clear that the majority of ingested lycopene is excreted in the feces and that 1000-fold more lycopene is absorbed and stored in the liver than accumulates in other target organs. Nonetheless, physiologically significant (nanogram) levels of lycopene are assimilated by key organs such as breast, prostate, lung, and colon, and there is a rough dose–response relationship between lycopene intake and blood levels. Pure lycopene was absorbed less efficiently than the lycopene-rich tomato carotenoid oleoresin and blood levels of lycopene in rats fed a grain-based diet were consistently lower than those in rats fed lycopene in a casein-based diet. The latter suggests that the matrix in which lycopene is incorporated is an important determinant of lycopene uptake. A number of issues remain to be resolved before any definitive conclusions can be drawn concerning the anticancer effects of lycopene. These include the following: the optimal dose and form of lycopene, interactions among lycopene and other carotenoids and fat soluble vitamins such as vitamin E and D, the role of dietary fat in regulating lycopene uptake and disposition, organ and tissue specificity, and the problem of extrapolation from rodent models to human populations. Exp Biol Med 227:864–868, 2002

Key words: tomato carotenoids; preclinical studies; cancer prevention; animal models

[1] To whom requests for reprints should be addressed at American Health Foundation, 1 Dana Road, Valhalla, NY 10595. E-mail: Leonard_cohen@nymc.edu; biolen@earthlink.net

1535-3702/02/22710-0864$15.00

Several retrospective and prospective epidemiological studies indicate that tomato consumption (1–3), lycopene intake (4, 5), and serum lycopene (6, 7) levels are associated with decreased risk of cancers, most notably prostate and lung cancer. These epidemiological leads have stimulated a number of animal model and cell culture studies designed to test this hypothesis and to establish its biological plausibility.

As of the present, eight animal model studies have been published (Table I). Positive results have been reported in mouse lung (8), rat urinary bladder (9), and rat colon cancer models (10). In addition, positive results have been reported in rat aberrant colon crypt formation (11, 12) and the rat hepatic preneoplasia model (13). However, with regard to mammary tumorigenesis, results have proven ambiguous. Supplementing a semipurified diet with extremely low levels $5 \times 10^{-5\%}$ (0.5 ppm) of pure lycopene, Nagasawa *et al.* (14) reported significant inhibition of spontaneous mammary tumor development. Using the dimethylbenz (a) anthracene (DMBA) model, Sharoni *et al.* (15) reported that intraperitoneal (ip) injections (10 mg/kg bw, twice per week) of a tomato carotenoid mixture 2 weeks before DMBA administration to termination resulted in the inhibition of tumor multiplicity but had no effect on tumor incidence, volume, or latency. Cohen *et al.* (16), using the *N*-nitrosomethylurea (NMU) model, reported no inhibitory effect on any parameter of tumor growth when a lycopene-rich tomato carotenoid oleoresin (TCO) or pure lycopene was fed in the diet to rats 250 and 500 ppm (mg/kg) throughout the experimental period. The discrepancies between the two latter studies could be due to different routes of administration (ip injection, versus diet) and/or differences in carcinogens used (host-activated DMBA versus direct-acting NMU), and strain differences (outbred Sprague–Dawley [SD] versus inbred F-344). It is of interest that our null result in the rat model are supported by three prospective human cohort studies by Howe *et al.* (17), Kushi *et al.* (18), and most recently by Smith-Warner *et al.* (19), which showed no association between intake of fruits and vegetables and breast cancer risk. A major problem with the published animal model studies is that no two are compa-

Table I. Lycopene and Cancer Animal Model Studies

Model	Investigators	Journal, year, and reference
(1) Mouse lung cancer	Kim *et al.*	Cancer Lett 1997 (8)
(2) Rat urinary bladder cancer	Okajima *et al.*	Jpn J Cancer Res 1998 (9)
(3) Rat colon cancer	Narisawa *et al.*	Jpn J Cancer Res 1998 (10)
(4) Rat colon ACF	Narisawa *et al.*	Cancer Lett 1996 (11)
	Wargovich *et al.*	Carcinogenesis 2000 (12)
(5) Hepatic preneoplasia	Astorg *et al.*	Nutr Cancer 1997 (13)
(6) Spontaneous mouse mammary tumor	Nagasawa *et al.*	Anti Cancer Res 1995 (14)
(7) Rat mammary tumor	Sharoni *et al.*	Cancer Detect Prev 1997 (15)
(8) Rat mammary tumor	Cohen *et al.*	Nutr Cancer 1999 (16)

rable because in each case different lycopene preparations, doses, and routes of administration were used, including intra-rectal instillation gavage, drinking water (dissolved in DMSO) ip injection, as tomato juice, and in the from of 10% water-soluble lycopene-coated beadlets.

Only one of the earlier studies (14) analyzed tissue levels (colon) of lycopene to assure that physiologically meaningful concentrations were achieved. Therefore, in our laboratory we embarked on a study to determine the uptake and disposition of lycopene in various tissues and serum of F-344 rats before performing out the NMU tumor study (20). The study design was predicated on the hypothesis that analysis of the effect of the entire complement of tomato carotenoids on tumorigenesis was preferable to that of pure lycopene. The rationale for this was that epidemiological evidence suggests that tomato consumption rather than lycopene intake *per se* (1) is associated with decreased cancer risk and the fact that, in nature, lycopene is always found in association with other carotenoids (21). In addition, because lycopene is ingested in the diet and is absorbed by the small intestine and transported *via* chylomicrons (21), it was decided to incorporate the lycopene-rich TCO in the diet so as to mimic human exposure to lycopene more closely.

The carotenoids present in the tomato carotenoid concentrate used in this study consisted of lycopene, β-carotene, phytoene phytofluene, and a small amount of α and ζ-carotene and an oxidative metabolite of lycopene, 2,6 cyclolycopene-1,5-diol (Table II). Six groups of 20 F-344 rats (10 males/10 females) were fed AIN-76A diets containing lycopene at 1240, 496, 248, 124, 50, and 0 mg/kg diet for a period of 10 weeks. The dose ranged from a low of 280 to a high of 7000 μg lycopene/day. Lycopene concentrations in selected organs and blood were then analyzed by the method of Zhao *et al.* (20) using reversed-phase high-performance liquid chromatography. It was found that more than 50% of ingested lycopene was excreted in the feces. As expected, lycopene concentrations were highest in the liver (42–120 μg/g). However, physiologically relevant levels, similar to those found in humans (1–4, 6, 7) were detected in prostate (47–97 ng/g), lung (134–227 ng/g), and mammary gland (174–309 ng/g) and these levels varied in a dose-related fashion (Table III). Serum lycopene concentrations varied from 160 to 285 ng/ml but exhibited a nonlinear relationship to dose (Table IV). Similar results were recently reported by Ferreira *et al.* (22) in a feeding study in ferrets and F-344 rats.

In the above studies, we did not explore the issue of *cis-trans* isomerism. Lycopene is an acyclic hydrocarbon with 11 conjugated double bonds in the *all-trans* configuration (23). Interestingly, as Clinton (2) and others (see Boileau *et al.* this volume) have reported, although lycopene in food is found primarily in the *all-trans* form, a variety of *cis* forms are commonly found in tissues and serum. In a recent rat study (24) it was shown that as dietary *all-trans* lycopene levels were increased the relative proportion of *cis* lycopene increased, particularly the 5 *cis* isomer. Moreover, in androgen-depleted (castrated) rats, liver *cis*-lycopene levels rose 2-fold compared with intact rats (no other tissue or blood levels were affected), suggesting interactions between lycopene isomers and sex-steroids. At present, the biologi-

Table II. Carotenoid Composition of Lycopene-Rich Tomato Oleoresin[a]

Carotenoid	mg/g	Percent
Lycopene	1240	3.7
β-carotene	280	1.2
Phytofluene	70	0.3
Phytoene	60	0.28
2,6-Cyclolycopene-1,5-diol	2	0.04

[a] As 5.7% suspension in MCT (Cognis Corp., La Grangę, IL) After Zhao *et al.* (20).

Table III. Distribution of Lycopene in Rat Prostate and Mammary Gland Tissue[a,b]

Group	Lycopene in diet (ppm)	Prostate	Mammary gland
		ng/g wet wt	
1	1250	100 (17)[c]	320 (130)
2	500	95 (48)	200 (30)
3	250	50 (37)	215 (62)
4	125	52 (26)	229 (54)
5	50	47 (16)	174 (57)
6	0	0	0

[a] Dose–response trend: prostate $P < 0.002$; breast, $P < 0.09$
[b] Lycopene fed as tomato carotenoid concentrate in AIN-76A diet. After Zhao *et al.* (20)
[c] Mean (SD).

Table IV. Rat Serum Lycopene Levels[a,b]

	In diet (ppm)	Serum[c]		
		Mean (SD)	Median	Range
Control	–	–	–	–
Lycopene	250	6 (4)[d]	7	0–10
Lycopene	500	10 (3)	12	6–13
Tomato carotenoids	250	63 (14)	60	47–85
Tomato carotenoids	500	94 (30)	87	58–148

[a] Female SD rats fed lycopene in Teklad (7001) grain-based diet
[b] Female F-344 rats fed lycopene-rich TCO in casein-based diet, mean (SD) = 170 (42) After Zhao *et al.* (20).
[c] ngs/ml
[d] N-6

cal significance of lycopene *cis* isomer accumulation in blood or tissue remains unknown.

Interestingly, in rats consuming the lycopene-rich TCO, levels of α-tocopherol and retinol in liver were elevated in a dose-related fashion along with lycopene (Table V). Alpha-tocopherol is known to be present in tomatoes (1); hence, the 3-fold increase in α-tocopherol levels in this study could be due to the increased intake of α-tocopherol in the supplemental group or to selective absorption and deposition in the presence of tomato carotenoids. The striking 10-fold increase in liver retinol concentrations (high dose versus controls) may be due to the β-carotene present in the tomato oleoresin but may also be due to selective absorption of β-carotene resulting from the presence of lycopene followed by enzymatic cleavage of β-carotene to retinol by dioxygenase. These findings underline the importance of interactions between the different carotenoid components of tomatoes, that may have important implications with regard to cancer prevention and that would not have been seen in studies using isolated lycopene.

Several intervening variables were inadvertently discovered as a result of this experiment. The tumor study was conducted using the out-bred SD rat fed a 4% corn oil, grain-based diet (Teklad 7001). However, the feeding study was performed using the in-bred F-344 rat fed casein-based semi-purified AIN-76A diet (5% corn oil). It was found that

Table V. Lycopene, Retinol, and α-Tocopherol Levels in Rat Liver (μg/g)[a–c]

Group	Lycopene in diet (ppm)	Lycopene	Retinol	α-Tocopherol
1	1250	120	1300	47
2	500	64	770	38
3	250	66	860	39
4	125	49	890	46
5	50	22	555	32
6	0	0	120	13

[a] Six Female F-344 rat livers pooled/group
[b] Dose–respone trend: $P < 0.01$ for lycopene, retinol, and α-tocopherol
[c] Lycopene fed as lycopene-rich TCO in AIN-76 diet After Zhao *et al.* (20).

female F-344 rats fed the lycopene-rich TCO at 500 ppm in the diet for 10 weeks exhibited significantly higher levels of serum lycopene (170 ± 42 ng/ml) than female SD rats fed the same amount of lycopene for 18 weeks (94 ± 30; $P < 0.05$; Table IV). This difference could be due to the longer feeding period but it is more likely due to strain-specific differences in absorption or differences in the diets used. For example, the grain-based (Teklad) diet contains a wide variety of plant-based components that are not present in the AIN-76A diet and has 20% less fat (4 vs 5%), which could influence the absorption of the highly lipophilic carotenoids (21). Another intriguing observation in the tumor study was that pure lycopene, incorporated at the same levels as the tomato carotenoid mixture in the diet, resulted in significantly lower serum levels of lycopene. The reasons for this finding are unclear; however, the result could be due to the fact that lycopene, in the presence of other carotenoids, may be absorbed more efficiently than in the absence of other carotenoids (25).

In vitro studies, despite their relatively artificial nature, provide valuable insights into the mechanisms by which carotenoids such as lycopene exert their cellular and intracellular effects. Cell culture studies have shown that lycopene inhibits neoplastic cell growth in lung (26, 27), breast (26), prostate (28), melanoma (29), and HL-60 cells (30–32). Various mechanisms have been proposed to explain the inhibitory effects of lycopene, including cell cycle arrest via insulin growth factor 1 signaling (33), enhanced gap-junction communication (34), induction of apoptosis, and EGF receptor downregulation (35, 36) and differentiation (31, 37) but none has been proven definitively. Cell culture studies have the disadvantage that the concentrations of lycopene used are often supraphysiological and that in vitro the O_2 partial pressures are higher than under *in vivo* conditions, which may influence the anti-oxidant properties of lycopene. Also, because transformed neoplastic cells are used *in vitro*, such studies provide little insight into the chemopreventive effects of lycopene. (One exception to this is the report by Bertram *et al.* (38), which demonstrated that lycopene inhibited *in vitro* neoplastic cell transformation.) Another problem with *in vitro* approaches lies in the extreme lipophilicity of lycopene. Most studies require either DMSO or tetrahydrofuran as solvents, both of which can be highly toxic in themselves to cultured cells. The use of water-soluble formulations such as cyclodextrans (cyclic carbohydrates which form water-soluble complexes with organic molecules), and water-soluble beadlets may provide a solution to this methodological problem.

In summary, animal model studies, although generally supportive of the anti-cancer activity of lycopene, have brought to light several important methodological issues relevant to future studies on the chemopreventive effects of lycopene. These include strain-specific and diet-specific effects, differences in uptake and distribution depending on whether lycopene is incorporated in the diet as pure lycopene or as a tomato carotenoid mixture, influences of to-

mato carotenoid supplements on the uptake and storage of other components of tomatoes or their metabolites such as β-carotene/retinol and α-tocopherol, and differential effects resulting from varying routes, doses, and methods of administration. Not considered in any detail this discussion, but worth noting with regard to future studies, is the possible contribution of other antioxidants present in tomatoes including vitamin C (160–240 mg/kg) and vitamin E (5–20 mg/kg; 39) and a variety of flavonoids such as naringenin, and phenolic acids such as chlorogenic acid (40).

Despite the problems associated with extrapolating from animal models to humans, controlling for these variables in future feeding studies should provide more definitive answers to the question of the anticancer effects of lycopene. Lastly, animal model studies, together with epidemiologic observations, suggest that tomato consumption may have organ-specific chemopreventive effects with lycopene exerting protective effects on prostate and lung but not on breast cancer.

1. Giovannucci E. Tomatoes, tomato-based products, lycopene, and cancer: Review of the epidemiologic literature. J Natl Cancer Inst **87:**1767–1776, 1995.
2. Clinton SK. Lycopene: Chemistry, biology, and implications for human health and disease. Nutr Rev **56:**35–51, 1998.
3. Rao AV, Agarwal S. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: A review. Nutr Res **19:**305–323, 1999.
4. Kristal AR, Cohen JH. Invited commentary: Tomatoes, lycopene, and prostate cancer. How strong is the evidence? Am J Epidemiol **151:**124–127, 2000.
5. Michaud DS, Feskanich D, Rimm EB, Colditz GA, Speizer FE, Willett WC, Giovannucci E. Intake of carotenoids and risk of lung cancer in 2 prospective US cohorts. Am J Clin Nutr **72:**990–997, 2000.
6. Casso D, White E, Patterson RE, Agurs-Collins T, Kooperberg C, Haines PS. Correlates of serum lycopene in older women. Nutr Cancer **36:**163–169, 2000.
7. Freeman VL, Meydani M, Yong S, Pyle J, Wan Y, Arvizu-Durazo R, Liao Y. Prostatic levels of tocopherols, carotenoids and retinol in relation to plasma levels and self-reported usual dietary intake. Am J Epidemiol **151:**109–118, 2000.
8. Kim DJ, Takasuka N, Kim JM, Sekine K, Ota T, Asamoto M, Murakoshi M, Nishino H, Nir Z, Tsuda H. Chemoprevention by lycopene of mouse lung neoplasia after combined initiation treatment with DEN, MNU, and DMH. Cancer Lett **120:**15–22, 1997.
9. Okajima E, Tsutsumi M, Ozono S, Akai H, Denda A, Nishino H, Oshima S, Sakamoto H, Konishi Y. Inhibitory effect of tomato juice on rat urinary bladder carcinogenesis after N-butyl-N-(4-hydroxybutyl) nitrosamine initiation. Jpn J Cancer Res **89:**22–29, 1998.
10. Narisawa T, Fukaura Y, Hasebe M, Nomura S, Oshima S, Sakamoto H, Inakuma T, Ishiguro Y, Takayasu J, Nishino H. Prevention of N-methylnitrosourea-induced colon carcinogenesis in F-344 rats by lycopene and tomato juice rich in lycopene. Jpn J Cancer Res **89:**1003–1008, 1998.
11. Narisawa T, Fukaura Y, Hasebe M, Ito M, Aizawa R, Murakoshi M, Uemura S, Khachik F, Nishino H. Inhibitory effects of natural carotenoids, α-carotene, β-carotene, lycopene and lutein, on colonic aberrant crypt foci formation in rats. Cancer Lett **107:**137–142, 1996.
12. Wargovich MJ, Jimenez A, McKee K, Steele VE, Velasco M, Woods J, Price R, Gray K, Kelloff GJ. Efficacy of potential chemopreventive agents on rat colon aberrant crypt formation and progression. Carcinogenesis **21:**1149–1155, 2000.
13. Astorg P, Gradelet S, Berges R, Suschetet M. Dietary lycopene decreases the initiation of liver preneoplastic by diethylnitrosamine in the rat. Nutr Cancer **29:**60–68, 1997.
14. Nagasawa H, Mitamura T, Sakamoto S, Yamamoto K. Effects of lycopene on spontaneous mammary tumour development in SHN virgin mice. Anticancer Res **15:**1173–1178, 1995.
15. Sharoni Y, Giron E, Rise M, Levy J. Effects of lycopene-enriched tomato oleoresin on 7,12-dimethylbenz[a]anthracene-induced rat mammary tumors. Cancer Detect Prev **21:**118–123, 1997.
16. Cohen, LA, Zhao, Z, Pittman, B, Khachik, F. Effect of dietary lycopene on N-methylnitrosourea-induced mammary tumorigenesis. Nutr Cancer 34:153–159, 1999.
17. Howe GR, Hirohata T, Hislop TG, Iscovich JM, Yuan JM, Katsouyanni K, Lubin F, Marubini E, Modan B, Rohan T. Dietary factors and risk of breast cancer: Combined analysis of 12 case-control studies. J Natl Cancer Inst **82:**561–569, 1990.
18. Kushi LH, Fee RM, Sellers TA, Zheng W, Folsom AR. Intake of vitamins A, C, and E and postmenopausal breast cancer. The Iowa Women's Health Study. Am J Epidemiol **144:**165–174, 1996.
19. Smith-Warner SA, Spiegelman D, Yaun SS, Adami HO, Beeson WL, van den Brandt PA, Folsom AR, Fraser GE, Freudenheim JL, Goldbohm RA, Graham S, Miller AB, Potter JD, Rohan TE, Speizer FE, Toniolo P, Willett WC, Wolk A, Zeleniuch-Jacquotte A, Hunter DJ. Intake of fruits and vegetables and risk of breast cancer: a pooled analysis of cohort studies. JAMA **285:**769–776, 2001.
20. Zhao Z, Khachik F, Richie JP Jr, Cohen LA. Lycopene uptake and tissue disposition in male and female rats. Proc Soc Exp Biol Med **218:**109–114, 1998.
21. Stahl W, Sies H. Lycopene: A biologically important carotenoid for humans? Arch Biochem Biophys **336:**1–9, 1996.
22. Ferreira AL, Yeum KJ, Liu C, Smith D, Krinsky NI, Wang X-D, Rusell RM. Tissue distribution of lycopene in ferrets and rats after lycopene supplementation. J Nutr **130:**1256–1260, 2000.
23. Stahl W, Schwartz W, Sundquist AR, Sies H. Cis-trans isomers of lycopene and β-carotene in human serum and tissues. Arch Biochem Biophys **294:**173–177, 1992.
24. Bioleau TWM, Clinton SK, Erdman JW. Tissue lycopene concentrations and isomer patterns are affected by androgen status and dietary lycopene concentration in male F344 rats. J Nutr **130:**1613–1618, 2000.
25. Van den Berg H. Carotenoid interactions. Nutr Rev **57:**1–10, 1999.
26. Levy J, Bosin E, Feldman B, Giat Y, Miinster A, Danilenko M, Sharoni Y. Lycopene is a more potent inhibitor of human cancer cell proliferation than either α-carotene or β-carotene. Nutr Cancer **24:**257–266, 1995.
27. Prakash P, Jackson CL, Gerber LE. Subcellular accumulation of β-carotene and retinoids in growth-inhibited NCI-H69 small cell lung cancer cells. Nutr Cancer **34:**76–82, 1999.
28. Pastori M, Pfander H, Boscoboinik D, Azzi A. Lycopene in association with alpha-tocopherol inhibits at physiological concentrations proliferation of prostate carcinoma cells. Biochem Biophys Res Commun **250:**582–585, 1998.
29. Hazuka MB, E-Prasad J, Newman F, Kinzie JJ, Parsad KN. β-carotene induces morphological differentiation and decreases adenylate cyclase activity in melanoma cells in culture. J Am Coll Nutr **9:**143–149, 1990.
30. Amir H, Karas M, Giat J, Danilenko M, Levy R, Yermiahu T, Levy J, Sharoni Y. Lycopene and 1,25-dihydroxyvitamin D3 cooperate in the inhibition of cell cycle progression and induction of differentiation in HL-60 leukemic cells. Nutr Cancer **33:**105–112, 1999.
31. Gross MD, Bishop TD, Belcher JD, Jocobs DR Jr. Induction of HL-60 cell differentiation of carotenoids. Nutr Cancer **27:**169–173, 1997.
32. Bertram JS. Carotenoids and gene regulation. Nutr Rev **57:**182–191, 1999.
33. Karas M, Amir H, Fishman D, Danilenko M, Segal S, Nahum A,

Koifmann A, Giat Y, Levy J, Sharoni Y. Lycopene interferes with cell progression and insulin-like growth factor I signaling in mammary cancer cells. Nutr Cancer **36:**101–111, 2000.

34. Zhang L-X, 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 **12:**2109–2114, 1991.
35. Palozza P, Maggiano N, Calviello G, Lanza P, Piccioni E, Ranelletti FO, Bartoli GM. Canthaxanthin induces apoptosis in human cancer cell line. Carcinogenesis **19:**373–376, 1998.
36. Muto Y, Fujii J, Shidoji Y, Moriwaki H, Kawaguchi T, Noda T. Growth retardatjon in human cervical-derived cell lines by β-carotene though down-regulation of epidermal growth factor receptor. Am J Clin Nutr **62:**1535S–1540S, 1995.
37. Biesalski H-K, Schaffer M. Comparative assessment of the activity of beta-carotene, retinoyl-beta-D-glucuronide and retinoic acid on growth and differentiation of a human promyelocytic leukemic cell line HL-60. Int J Vitam Nutr Res **67:**357–363, 1997.
38. Bertram JS, Pung A, Churly M, Kappock TJ, Wilkins SR, Cooney RV. Diverse carotenoids protect against chemically induced neoplastic transformation. Carcinogenesis **12:**671–678, 1991.
39. Holland B, Welch AA, Unwin ID, Buss DH, Paul AA, Southgate DAT. The Composition of Foods, Fifth revised and extended edition. McCance and Widdowson's, UK 1992.
40. Davies JN, Hobson GE. Constituents of tomato fruit, the influence of environment, nutrition and genotype. Crit Rev Food Sci Nutr **15:**205–280, 1981.