Food Chemistry 132 (2012) 1156–1160

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of a hydrodynamic process on extraction of carotenoids from tomato

Betty K. Ishida *, Mary H. Chapman

Western Regional Research Center, Agricultural Research Service, United States Department of Agriculture, 800 Buchanan St., Albany, CA 94710, USA

ARTICLE INFO

Article history:
Received 24 March 2009
Received in revised form 31 October 2011
Accepted 8 November 2011
Available online 2 December 2011

Keywords:
Tomatoes
Lycopene
Cis- and *trans*-lycopene isomers
Phytoene
Phytofluene
Carotenoids
Bioavailability
Hydrodynamic

ABSTRACT

We evaluated the results of using a proprietary hydrodynamic method, which was introduced with the hope of increasing accessibility of beneficial nutrition-enhancing fruit and vegetable products. Tomato, a major dietary source of carotenoids, notably lycopene, was tested because of its many health benefits to consumers. Samples before and after treatment were compared for lycopene, phytoene, and phytofluene contents. Extractable lycopene and other carotenoids increased significantly. In nature, lycopene exists almost exclusively as the all-*trans* stereoisomer. *Cis*-lycopene isomers form during cooking and digestion, resulting in higher percentages in plasma and tissues than ingested. *Cis*-lycopene isomers are more bioavailable than all-*trans* lycopene. Extraction using this proprietary method increased extracted *cis*-lycopene to as high as 43% of the total lycopene, indicating increased isomerisation. This method could therefore contribute significantly to the delivery of health benefits of biologically available lycopene from tomato products for metabolic functions.

Published by Elsevier Ltd.

1. Introduction

Selection of fruits and vegetables commonly purchased by consumers was determined largely by seasonal and geographic availability; however, recent globalisation of the world's economy coupled with concurrent improvements in transportation and preservation of produce has severed previous constraints based upon growing season. Consumers are presented with a myriad of choices and, therefore, are now basing more and more of their decisions upon the potential health benefits of these products. The realisation that most of these potential health benefits may be attributed directly to secondary metabolites present in produce has resulted in efforts to develop methods to extract these compounds for inclusion in functional foods or methods to enhance their bioavailability in processed products. Tomato is among the many crops that have received considerable attention with interest being focused on the health benefits of its carotenoids.

Consumers have long valued tomato's bright red colour for its attractiveness and appeal. Lycopene, which is the carotenoid that is responsible for this bright red colour, is one of its major health-beneficial components. Among the carotenoids, it is the most powerful biological antioxidant (DiMascio, Kaiser, & Sies, 1989); therefore, its preservation in food is of great importance to human health. In fact, it is often added to processed foods to enhance both colour and health benefits. Several studies show that processing tomatoes changes the isomeric content of lycopene, increasing the amount of *cis*-lycopene isomers (Gartner, Stahl, & Sies, 1997; Ishida, Roberts, Chapman, & Burri, 2007; Nguyen, Francis, & Schwartz, 2001; Stahl & Sies, 1992) and that this change (e.g., see Fig. 1) enhances the bioavailability of lycopene in processed tomatoes because *cis* lycopene is more bioavailable than the *trans* isomer (Burri, Chapman, Neidlinger, Jung, & Ishida, 2008; Gartner et al., 1997; Stahl & Sies, 1992). Other investigators report that the *cis*-isomeric content of lycopene could be increased by heating between 75 and 100 °C, but not at 25–50 °C (Hackett, Lee, Francis, & Schwartz, 2004). Wang and Chen (2006) showed that a tomato powder having a maximum isomerisation (*cis*-isomer-lycopene content of 51.0–53.8%) could be produced using supercritical carbon dioxide at 350 bars and 80 and 90 °C, but the maximum yield of total lycopene was obtained at a lower temperature. They suggested the use of this method for possible commercial production of a highly active lycopene powder.

It was postulated that the proprietary method considered in this study (Duffield & Matula, 2010) could be used in conjunction with normal processing methods to increase available lycopene in processed tomatoes by increasing its release from tomato tissues and increasing its *cis*-isomeric content of lycopene. (Preliminary results of treated tomato samples indicated a change in isomeric content of lycopene.) For these reasons, its complete extraction from food sources both for use as an additive and measurement of total lycopene content is of interest.

Natural products may be broadly divided into categories of water-soluble (e.g., phenolic compounds) and lypophilic, water-insoluble (e.g., lycopene) components, and considerable effort has been expended towards developing specific extraction

* Corresponding author. Tel.: +1 510 233 3936.
E-mail address: bkishida@gmail.com (B.K. Ishida).

0308-8146/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2011.11.036

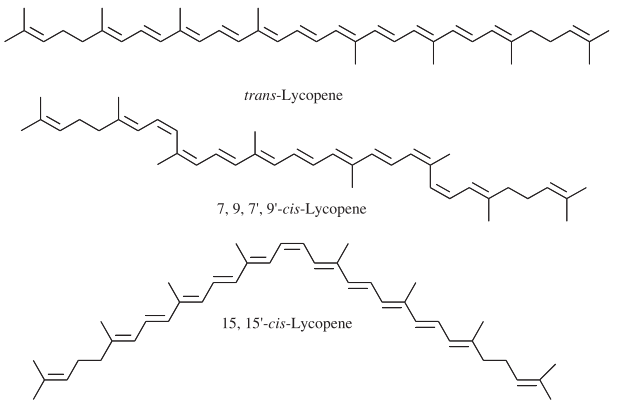

Fig. 1. Structures of *trans*-lycopene, *tetra-cis*-lycopene, and 15,15′-*cis*-lycopene.

methods for these two categories. Most extraction methods follow a common path consisting of release of desired compounds from their matrices by disrupting plant tissue, followed by clarification to remove unwanted cellular components, and an initial liquid–liquid or liquid–solid extraction before final purification. Because many mechanical processes typically employed to disrupt tissues (e.g., cutting, shearing, macerating, etc.) contribute to thermal or oxidative degradation of labile, biologically active molecules, alternative, gentler disrupting methods should be evaluated. One such method (Duffield & Matula, 2010) is the use of hydrodynamic methods to decrease the size of particles, while possibly altering the stereochemical form of the carotenoids. A possible mechanism leading to this conversion is cavitation (Suslick, 1990). Interest in the use of ultrasonic methods in biology has focused primarily on its application to open micropores in cells to deliver genes and therapeutic agents to specific cells. In the present study, we evaluated tomato samples that were treated by this proprietary hydrodynamic method to increase extractable carotenoids.

2. Experimental

2.1. Materials and chemicals

Solvents [acetonitrile, chloroform, ethanol (EtOH), methanol, HPLC-grade, ethyl lactate, methyl-*t*-butyl ether (MTBE), and ethyl acetate (EtOAc)], along with DL-α-tocopherol (TOC), perchloric acid (70%, ACS reagent), glacial acetic acid (ACS PLUS), and formic acid (88%, ACS reagent-grade) were purchased from Fisher Scientific, Ltd. (Waltham, MA). Dichloromethane, 99.9% HPLC-grade, DL-α-lipoic acid, anhydrous tetrahydrofuran (THF), 4-(dimethylamino) benzaldehyde (DMAB), and, for standard solutions, β-carotene (type IV from carrots), mixed isomer carotene (from carrots), and lutein (from alfalfa) were obtained from Sigma–Aldrich (St. Louis, MO). Water was deionised to ⩾18.1 MΩ/cm resistance, using a Barnstead NANOpure Deionisation System (Dubuque, IA), and filtered through a 0.45-μm, type HA membrane filter (Millipore, Billerica, MA) before use. Strata-X solid phase extraction (SPE) columns (30 mg/1 ml) were purchased from Phenomenex (Torrance, CA). Lycopene for standard solutions was extracted and purified from berries of autumn olive (*Elaeagnus umbellate* Thunberg) plants, which were a gift from Beverly A. Clevidence (Beltsville Human Nutrition Research Center, USDA, ARS, Beltsville, MD). Treated tomato samples were provided by CRS Technologies, Inc. (Wilmington, DE). These samples were generated using CRS's proprietary system (Duffield & Matula, 2010). Tomato samples were obtained from a tomato processing plant.

2.2. Carotenoid extraction

Dry weights of tomato preparations were determined using a Model AVC-80 microwave moisture/solids analyser (CEM Corp., Mathews, NC). Samples (2–4 g) were placed between two tared fibreglass pads and heated at 50% power for 4.5 min, which was sufficient time for water loss from the sample to be complete. Moisture content (or percent solids) was determined by difference in weight after drying. Lyophilised tomato samples, each weighing 0.25–1.0 g, were placed in 20-ml glass vials along with antioxidant where specified, and 10 ml of ethyl acetate was added. In subdued light, vials were capped, mixed vigorously with the aid of a vortex mixer, and placed in a temperature-controlled water bath kept at 60 °C. After 2 h, 500 μl of mixture was removed, dried under a stream of N_2 gas, resuspended in THF, passed through a 0.2-μm nylon filter, and injected into the HPLC. Alternatively, an aliquot of extracted sample was filtered and injected into the HPLC directly. When specified, samples were taken after 1 and 2 h of extraction and treated as above. For comparison, carotenoids were also extracted from tomato preparations, using dichloromethane/methanol/H_2O (40/40/20, v/v/v) by the method described by Ishida, Ma, and Chan (2001) with modifications to maximise extraction of both polar and nonpolar species (Ishida, Ma, Chan, Bartley, & Grossman, 2001). Samples were analysed in triplicate and standard deviations calculated. In some cases when large numbers of samples were analysed, e.g., Table 1, relative standard deviations based

Table 1
Carotenoid contents of tomato samples untreated and treated using the proprietary hydrodynamic process. Samples were extracted using ethyl acetate and analysed using our HPLC method. Results are expressed as μg lycopene/g dry weight sample.

Tomato sample	*Trans*	*Cis*	Total	% *Cis*-		
	Lycopene	Lycopene	Lycopene	Lycopene	Phytoene	Phytofluene
First extraction[a]						
VFNT Cherry	119 (5.25)[b]	10 (9.83)	129 (6.87)	8	0	0
Control	188 (8.16)	19 (9.98)	207 (9.45)	9		
Treated	335 (6.89)	159 (8.16)	494 (7.62)	32	nd[c]	91
Second extraction[a]						
VFNT Cherry	153 (1.88)	50 (9.21)	203 (9.62)	25	0	50
Control	147 (4.91)	132 (9.21)	274 (8.80)	47	0	69
Treated	138 (7.56)	192 (9.32)	329 (7.63)	58	72	35
Total extracted						
VFNT Cherry	272 (3.82)	61 (9.25)	333 (8.25)	18	0	139
Control	335 (6.58)	150 (9.28)	485 (9.14)	31	nd	165
Treated	473 (7.223)	351 (8.84)	823 (7.60)	43	nd	163

[a] Each extraction was performed for 1 h at 60 °C.
[b] Relative standard deviation.
[c] No data.

on three separate, random samples were calculated, using the equation:

$$\text{Relative standard deviation} = \text{standard deviation}/(\text{average value}) \times 100.$$

2.3. Carotenoid analysis

HPLC analysis was carried out using a Waters HPLC equipped with a model 2690 Separations Module, model 996 Photodiode Array Detector, and a C_{30} carotenoid column (4.6 × 240 mm i.d., 3-μm particle diameter, polymeric) (Waters Corp., Milford, MA). Carotenoids were analysed essentially according the method described by Ishida and Chapman (2004), except that a mobile phase of MTBE/ MeOH/EtOAc (45/40/15, v/v/v) was used. *Cis*- and *trans*-lycopene isomers were identified by their absorption spectra and retention times (Ishida & Chapman, 2004; Ishida et al., 2007). Lycopene used for standard solutions (see above under Section 2.1) was analysed by HPLC and found to be 97% *trans* lycopene. No standards were available for the various *cis*-lycopene stereoisomers. Only *tetra-cis* lycopene could be identified because of its distinctive absorption spectrum, but other specific *cis* isomers of lycopene could not be distinguished from one another. However, because we used a similar mobile phase and the same column as those used by Fröhlich, Conrad, Schmid, Breithaupt, and Böhm (2007), we assumed that the order of elution of the *cis*-lycopene isomers was also similar. Both *trans* and *cis* isomers of lycopene were quantified by the all-*trans* lycopene calibration as in other studies (Fröhlich et al., 2007; Ishida, Turner, Chapman, & McKeon, 2004; Ishida et al., 2007). *Cis*-lycopene isomers, other than *tetra-cis* lycopene, were grouped together for quantification. (No *tetra-cis* lycopene was detected in our samples, which is the usual case with the exception of Tangerine tomatoes.)

3. Results and discussion

Samples of tomato that had been treated by the proprietary method were received for evaluation by analyses of tomato carotenoids. This treatment was believed to result in increased accessibility of valuable phytonutrients present in the fruit, possibly by releasing them from membrane entrapment by disruption or solubilisation.

3.1. Effects of the proprietary process on tomato

We received tomato preparations that were treated at a tomato processing plant: one group of samples had been treated using the proprietary process, and others without treatment were used as control samples. In addition to these samples, as another control, we analysed carotenoids of tomatoes grown in our own greenhouse, *Solanum lycopersicon* (formerly *Lycopersicon esculentum*) cv. VFNT Cherry, which were frequently used in our previous tomato studies (Ishida & Chapman, 2009; Ishida et al., 2001; Ishida, Ma, Chan, Bartley, et al., 2001; Ishida et al., 2007). Samples were extracted using ethyl acetate, as described above, and analysed for carotenoids, including lycopene-isomer profile. Data obtained on lycopene-isomer content are shown in Table 1. Note that a much larger amount of total lycopene was extracted from treated tomato samples than untreated samples (control and VFNT). In addition, a much larger percentage of *cis*-lycopene isomers was obtained from the treated samples. The second extraction yielded a larger percentage of *cis* isomers from untreated samples than the first extraction. The first extraction increased the yield of *cis*-lycopene isomers 8.4-fold and the total amount of lycopene by 2.4-fold. Although the increased amount of lycopene extracted after treatment cannot be attributed unequivocally to increased accessibility or isomeric conversion, it seems reasonable to assume that it is a result of a combination of the two, accessibility plus conversion of some all-*trans* isomers to *cis* isomers. Samples were extracted for 2 h at 60 °C. As reported recently (Ishida & Chapman, 2009), considerable isomerisation of all-*trans* lycopene to *cis*-lycopene isomers occurs at this temperature, the amount depending upon the solvent. Oxidation also occurs. However, the fact that the total amounts of both *trans* lycopene and total lycopene extracted after 2 h increase (Table 2) indicates increased lycopene accessibility resulting from the proprietary treatment. The extraction process apparently increases isomerisation in the untreated sample, because the percentage of *cis* isomers obtained in the second extraction is much larger than from the first, i.e., the amount of *cis* isomers increases with time. Because the solvent used, ethyl acetate, is a polar solvent, one would not expect that the *cis* isomers would be more resistant to extraction than the non-polar *trans* isomer of lycopene.

A second set of samples was obtained from the same source: one was a sample of lye peel from processing tomatoes and the other a powder produced from whole tomatoes. Both samples were treated using the proprietary method. These samples were extracted, using ethyl acetate as the solvent with and without the addition of α-tocopherol (TOC). After extraction, (1) an aliquot of the extract was injected directly onto the HPLC column or (2) the extract was evaporated to dryness under a stream of nitrogen, resuspended in THF, then analysed by HPLC. These data are shown in Table 2.

In all cases, the percentage of *cis*-lycopene isomers is higher in treated tomato samples than found in extracts that were not treated using the proprietary method (see Table 1). Depending upon the addition of the antioxidant TOC or addition and/or avoidance of drying the extract, the percentage of *cis*-lycopene

Table 2
Carotenoid content (μg/g DW) of preparations of lye-peeled tomatoes and of whole tomato extracted using ethyl acetate both treated using the hydrodynamic process. Two methods were followed: aliquots of extracts were either injected into the HPLC system directly or evaporated to dryness, resuspended in THF, and then injected into the HPLC.

Tomato sample	*Trans*-lycopene	*Cis*-lycopene	Total lycopene	% *Cis*-lycopene	Phytoene	Phytofluene	Lutein	Carotenes
Lye peel −TOC[a]	667 (110)[b]	227 (42)	894 (152)	25	2064 (500)	148 (21)	99 (75)	96 (119)
+TOC	769 (84)	252 (48)	1021 (84)	25	529 (174)	78 (110)	36 (9)	14 (19)
−TOC + THF[a]	170 (4)	186 (3)	356 (7)	52	1178 (500)	14 (19)	51 (9)	0 (0)
+TOC + THF	135 (76)	185 (39)	320 (129)	58	280 (12)	0 (0)	29 (0)	0 (0)
Whole tomato −TOC	2461 (261)	873 (84)	3334 (26)	26	5717 (3096)	1157 (33)	56 (79)	649 (62)
+TOC	2643 (305)	976 (62)	3619 (368)	27	1043 (1476)	661 (935)	0 (0)	776 (79)
+THF	588 (69)	676 (103)	1264 (172)	53	6747 (5134)	180 (255)	245 (19)	0 (0)
+TOC + THF	686 (11)	788 (1)	1473 (11)	53	452 (639)	177 (250)	253 (6)	0 (0)

[a] TOC, α-tocopherol; THF, tetrahydrofuran.
[b] (), Standard deviation.

isomers ranged from approximately 25–58%; whereas, extracts of untreated tomato samples contained 8–9% *cis* lycopene. Also, in all cases, drying the extract decreased amounts of both *trans*- and *cis*-lycopene isomers, and the addition of TOC protected carotenoids from degradation by oxidation. Extraction of lutein, phytoene, and phytofluene (Table 2), however, was greater in the absence of TOC, which apparently interacts with the carotenoids and interferes with their extraction. We see an exception in the case of extraction of phytofluene and lutein from whole tomato powder that was dried down and resuspended in THF (see Table 2). In this case, the effect of TOC is not apparent.

The data in Table 2 show higher phytoene and phytofluene concentrations than we have seen in our previous analyses of tomato products, typically as seen in Table 1 in the VFNT Cherry tomato and control (untreated) samples. Our HPLC system is not optimised for separating and analysing these two carotenoids; however, it does provide an estimate. The values obtained for all of the treated samples in Table 2 are significantly higher than those found in typical tomato products, such as the two in Table 1. This suggests that the proprietary method releases phytoene and phytofluene from the tomato matrix.

3.2. Assessment of the benefits of the proprietary method to tomato

The protective effects of carotenoids against cardiovascular and heart disease, epithelial cell cancers, and degenerative eye disease, such as cataracts and age-related macular degeneration, are attributed to their antioxidant properties and role as free radical scavengers. In nature lycopene is present primarily in its *trans*-isomeric form. Recently the importance of the stereoisomeric form of lycopene to its absorption during digestion of foods has been established. Several investigations show that *cis* isomers of lycopene are more bioavailable than its all-*trans* isomer (Boileau, Merchen, Wasson, Atkinson, & Erdman, 1999; Burri, et al., 2008; Clinton et al., 1996; Schierle et al., 1997; Stahl & Sies, 1992; Unlu, Bohn, Francis, Clinton, & Schwartz, 2007). Another study compared the *in vitro* digestive stability, efficiency of micellerisation, and uptake and stability in Caco-2 intestinal cells and concluded that *cis*-lycopene isomers are more bioaccessible than the all-*trans* isomer (Failla, Chitchumroonchokchai, & Ishida, 2008). Therefore, foods containing higher amounts of *cis*-lycopene isomers would provide greater absorption of lycopene than those having high *trans*-lycopene contents.

Cis isomers of lycopene can also be formed by thermal- (Hackett et al., 2004) or photoisomerisation and reactions catalysed by acid or iodine (Zechmeister, LeRosen, Went, & Pauling, 1941). In another investigation (Unlu et al., 2007) to compare bioavailability of lycopene isomers, a tomato sauce rich in *cis* lycopene was prepared by additional heating of a conventional tomato sauce rich in the all-*trans*-lycopene isomer. The first sauce contained 95% *trans* lycopene, and the *cis*-isomer-rich sauce contained only 55% all-*trans* lycopene. Alternatively, a high *cis*-lycopene tomato powder has been prepared from tomato pulp, using supercritical fluid carbon dioxide at 350 bars and 70 °C (Wang & Chen, 2006). In this tomato powder, *cis*-lycopene isomers comprise 41.4% of the total lycopene.

Comparing the amounts of *cis*-lycopene isomers obtained using the proprietary method (Duffield & Matula, 2010) with heat treatment of tomato oleoresin (Hackett et al., 2004) and supercritical fluid treatment plus heat (Wang & Chen, 2006), similar percentages of *cis*-lycopene isomers are obtained. Heat treatment produced a sauce containing about 45% *cis* isomers, and the supercritical CO_2 treatment plus heat produced a tomato powder containing 41.4% *cis* lycopene. Table 1 shows that the treated tomato samples in our study contained 43% *cis*-lycopene isomers. It seems that this hydrodynamic process might provide a more energy-efficient and therefore less costly method of producing a high *cis*-lycopene food source, which promises greater health benefits to humans. Note: This process has not yet been optimised for maximum carotenoid extraction/isomerisation.

4. Conclusion

This proprietary hydrodynamic method of treating vegetable matter provides increased accessibility of carotenoids to extraction procedures on tomato, while changing the stereoisomeric profile of lycopene to one that is more bioavailable and therefore more beneficial to consumers.

Acknowledgements

We are grateful to CRS Technologies, Inc. (Wilmington, DE) for providing samples of tomato for analyses. We also thank Thomas A. McKeon and Gary R. Takeoka (Western Regional Research Centre, Agricultural Research Service, U.S. Department of Agriculture) for their careful reading of the manuscript and helpful comments and Tom Matula (University of Washington) for his helpful and insightful suggestions.

References

Boileau, A. C., Merchen, N. R., Wasson, K., Atkinson, C. A., & Erdman, J. W. Jr., (1999). Cis-lycopene is more bioavailable than trans-lycopene in vitro and in vivo in lymph-cannulated ferrets. *Journal of Nutrition, 129*(6), 1176–1181.

Burri, B. J., Chapman, M. H., Neidlinger, T. R., Jung, S. S., & Ishida, B. K. (2008). Tangerine tomatoes increase total and tetra-cis-lycopene isomer concentrations more than red tomatoes in healthy adult humans. *International Journal of Food Sciences and Nutrition, 10*, 1–16.

Clinton, S. K., Emenhiser, C., Schwartz, S. J., Bostwick, D. G., Williams, A. W., Moore, B. J., et al. (1996). Cis–trans lycopene isomers, carotenoids, and retinol in the human prostate. *Cancer Epidemiology, Biomarkers and Prevention, 5*(10), 823–833.

DiMascio, P., Kaiser, S., & Sies, H. (1989). Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Archives of Biochemistry and Biophysics, 274*, 1–7.

Duffield, R. B., & Matula, T. J. (2010). U.S. Provisional application serial no. 61/418,515, Method for processing plant material, December 1.

Failla, M. L., Chitchumroonchokchai, C., & Ishida, B. K. (2008). In vitro micellarization and intestinal cell uptake of cis isomers of lycopene exceed those of all-trans lycopene. *Journal of Nutrition, 138*(3), 482–486.

Fröhlich, K., Conrad, J., Schmid, A., Breithaupt, D. E., & Böhm, V. (2007). Isolation and structural elucidation of different geometrical isomers of lycopene. *International Journal for Vitamin and Nutrition Research, 77*(6), 369–375.

Gartner, C., Stahl, W., & Sies, H. (1997). Lycopene is more bioavailable from tomato paste than from fresh tomatoes. *American Journal of Clinical Nutrition, 66*, 116–122.

Hackett, M. M., Lee, J. H., Francis, D., & Schwartz, S. J. (2004). Thermal stability and isomerization of lycopene in tomato oleoresins from different varieties. *Journal of Food Science, 69*(7), 536–541.

Ishida, B. K., & Chapman, M. H. (2004). A comparison of carotenoid content and total antioxidant activity in catsup from several commercial sources in the United States. *Journal of Agricultural and Food Chemistry, 52*(26), 8017–8020.

Ishida, B. K., & Chapman, M. H. (2009). Carotenoid extraction from plants using a novel, environmentally friendly solvent. *Journal of Agricultural and Food Chemistry, 57*, 1051–1059.

Ishida, B. K., Ma, J., & Chan, B. (2001). A simple, rapid method for HPLC analysis of lycopene isomers. *Phytochemical Analysis, 12*(3), 194–198.

Ishida, B. K., Ma, J. C., Chan, B. C., Bartley, G. E., & Grossman, J. N. (2001). A modified method for simple, rapid HPLC analysis of lycopene isomers. *Acta Horticulturae, 542*, 235–242.

Ishida, B. K., Roberts, J. S., Chapman, M. H., & Burri, B. J. (2007). Processing tangerine tomatoes: Effects on lycopene-isomer concentrations and profile. *Journal of Food Science, 72*(6), C307–C312.

Ishida, B. K., Turner, C., Chapman, M. H., & McKeon, T. A. (2004). Fatty acid and carotenoid composition of Gac (Momordica cochinchinensis Spreng) fruit. *Journal of Agricultural and Food Chemistry, 52*(2), 274–279.

Nguyen, M., Francis, D., & Schwartz, W. (2001). Thermal isomerization susceptibility of carotenoids in different tomato varieties. *Journal of the Science of Food and Agriculture, 81*, 910–917.

Schierle, J., Bretzel, W., Buhler, I., Faccin, N., Hess, D., Steiner, K., et al. (1997). Content and isomeric ratio of lycopene in food and human blood plasma. *Food Chemistry, 59*(3), 459–465.

Stahl, W., & Sies, H. (1992). Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. *Journal of Nutrition, 122*(11), 2161–2166.

Suslick, K. S. (1990). Sonochemistry. *Science, 247*, 1439–1445.

Unlu, N. Z., Bohn, T., Francis, D., Clinton, S. K., & Schwartz, S. J. (2007). Carotenoid absorption in humans consuming tomato sauces obtained from tangerine or high-beta-carotene varieties of tomatoes. *Journal of Agricultural and Food Chemistry, 55*(4), 1597–1603.

Wang, C., & Chen, G. H. (2006). Tomato pulp as a source for the production of lycopene powder containing high proportion of cis-isomers. *European Food Research and Technology, 222*, 347–353.

Zechmeister, L., LeRosen, A. L., Went, F. W., & Pauling, L. (1941). Prolycopene, a naturally occurring stereoisomer of lycopene. *Proceedings of the National Academy of Sciences USA, 2*, 468–471.