Petition to Include Synthetic Crystalline LYCOPENE at 7 CFR 205.605

Item A

This petition seeks inclusion of **Synthetic Crystalline LYCOPENE** on the National List as a non-agricultural (non-organic) substance allowed in or on processed products labeled as "organic" or "made with organic (specified ingredients)," at §205.605(b).

Item B

1. The substance's chemical or material common name.

Lycopene is a naturally occurring aliphatic hydrocarbon of the carotenoid class. Lycopene is the most abundant carotenoid in ripe tomatoes and comprises 80-90% of the total pigment. Lycopene contains thirteen double bonds. The all-trans isomer is predominant in tomatoes and other natural sources. Storage, cooking, food processing, and exposure to light may result in some isomerization of the all-*trans* form to various *cis* forms including the 5-*cis*, 9-*cis*, 13-*cis*, and 15-*cis* forms.

Synthetic crystalline lycopene is predominantly the all *trans*-lycopene (>70%) with some 5-*cis*-lycopene and other *cis* isomers.

Other chemical names for lycopene are ***ψ,ψ***-carotene and (all-E)-all-*trans*-lycopene. It is commonly known as all-*trans*-lycopene. The systematic name for the all-*trans*-lycopene is (all-E)-2, 6, 10, 14, 19, 23, 27, 31-octamethyl-2,6, 8**,** 10, 12, 14, 16, 18, 20, 22, 24, 26, 30-dotricaconta-tridecaene.

2a. The name and address of the manufacturer/producer of the substance.

Synthetic crystalline lycopene is produced by:

BASF SE
Carl-Bosch-Straße 38
67056 Ludwigshafen/Germany

2b. The name, address and telephone number and other contact information of the petitioner.

International Formula Council
1100 Johnson Ferry Road NE, Suite 300
Atlanta, GA 30342
Contact: Mardi Mountford, Executive Vice President
Phone: (678) 303-3027
Email: mmountford@kellencompany.com

3. The intended or current use of the substance as a nonagricultural ingredient.

Synthetic lycopene provides an important carotenoid to infant formulas, ensuring that the nutritional quality of infant formula is close to that of human milk.

In conventional foods, synthetic lycopene is GRAS, for use as a direct food ingredient in breakfast cereals (ready-to-eat and cooked), drinks (juice drinks, energy drinks, and dairy fruit drinks), instant soup, low fat dressings, meal replacements, meatless meat products, nutrient bars, salty snacks, crackers and yogurt at levels ranging from 5 to 70 milligrams per kilogram of food.

4. A list of the handling activities for which the substance will be used. If used for handling (including processing), the substance's mode of action must be described.

Lutein and lycopene are among the most concentrated carotenoids in human tissue, including human milk (Khachik *et al*. 1997[1]; Schweigert *et al*. 2004[2]). Carotenoids are not synthesized in humans, therefore their deposition in these tissues is a consequence of consuming a varied and healthful diet of fruits and vegetables that are natural sources of the nutrients. Consumption of a diet rich in the fruits and vegetables that are sources of carotenoids is regarded as prudent and has been associated with decreased risk for certain cancers and eye disease in some but not all studies (Krinsky and Johnson 2005)[3]. The beneficial effect of carotenoids has been attributed in part to the antioxidant function of these substances, though other mechanisms of action are possible (Krinsky and Johnson 2005). Infants consuming human milk are exposed to carotenoids in early postnatal life, while infants consuming cow's milk-based formulas have low or no intake of these nutrients. The concentration of carotenoids in human milk is related to the mother's diet, and consequently exposure to carotenoids from human milk is highly variable across women. The limited data in the published literature show that carotenoid levels in term breast-fed infants are significantly higher than levels in infants fed infant formula (Bettler *et al*. 2009[4]; Mackey *et al*. 2008[5]**)**

[1] Khachik, F., C. J. Spangler, et al. (1997). "Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum." Anal Chem **69**(10): 1873-1881.
[2] Schweigert, F. J., K. Bathe, et al. (2004). "Effect of the stage of lactation in humans on carotenoid levels in milk, blood plasma and plasma lipoprotein fractions." Eur J Nutr **43**(1): 39-44.
[3] Krinsky, N. I. and E. J. Johnson (2005). "Carotenoid actions and their relation to health and disease." Mol Aspects Med **26**(6): 459-516.
[4] Bettler, J., J. P. Zimmer, et al. (2010). "Serum lutein concentrations in healthy term infants fed human milk or infant formula with lutein." Eur J Nutr **49**(1): 45-51.
[5] Mackey AD, Price PT, Oliver J, Albrecht D, Boileau A (2008). Relative bioavailabilitiy of carotenoids in infant formula and human milk. Presented at: Clinical Nutrition Week, February 13, Chicago IL [abstract]

5. The source of the substance and a detailed description of its manufacturing or processing procedures from the basic component(s) to the final product.

Synthetic lycopene is produced by the Wittig condensation of synthetic intermediates commonly used in the production of other carotenoids used in food. The three-stage process for this chemical synthesis of BASF is described in the GRAS determination (available as Appendix A). U.S. Patent No. 5,166,445, included in Appendix A, is one of the many descriptions of the preparation of lycopene alluded to in footnote 1 of the GRAS determination.

The current process has been modified as follows to lessen its environmental impact:

Stage one produces an organic solution of C_{15} phosphonium methanesulfonate in heptane, and stage two produces an organic solution of C_{10} dialdehyde in toluene. In stage three, the intermediates produced in stages one and two are gradually combined with sodium methoxide solution and undergo a condensation reaction to form crude lycopene. The crystallizing slurry is discharged to a process filter and the crystals are washed several times with water and methanol. In a further purification step the material is suspended in methanol. The mixture is centrifuged and the filter cake is washed with methanol. After the washing, the filter cake is dried with preheated nitrogen. The filter cakes of crystalline synthetic lycopene are stored and shipped in air-tight containers under inert gas.

6. A summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance.

Lycopene has not been reviewed by State or private organic certification programs. Lycopene has been added to infant formulas since 2009 following FDA review of submitted data as required by the Infant Formula Act of 1980.

7. Information regarding EPA, FDA, and State regulatory authority registrations, including registration numbers.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) specification for synthetic lycopene, identified as "INS 160d(i)," was prepared at the 67th JECFA in 2006 and published in FAO JECFA Monographs 3 (2006). Synthetic lycopene is permitted in the EU as food colorant as E 160d(i) and novel food ingredient. Purity requirements are included in the JEFCA specification and the Food Chemical Codex. Copies are included in Appendix B.

In a response dated April 7, 2005, the FDA stated that the agency had "no objection" to the self-affirmation of synthetic lycopene as GRAS filed by the BASF Corporation and identified as GRAS Notice GRN No. 119[6]. A copy of the FDA letter is available in Appendix A.

8a. The Chemical Abstracts Service (CAS) Registry Number for lycopene is 502-65-8.

8b. Labels of infant formulas containing the substance.

See Appendix C.

9a. The substance's physical properties

Lycopene forms long, deep red needles. One gram dissolves in 50 mL of carbon disulfide, in 3.1 liter of boiling ether, in 12 liters of boiling petroleum ether, and in 14 liters of hexane at 0°C. Lycopene is insoluble in water.

Because synthetic crystalline lycopene is unstable due to its sensitivity to oxygen (exposure results in rapid isomerization and oxidation), the crystalline material is stored under inert gas (nitrogen) or suspended in edible oils or formulated as cold-water dispersible powders or microencapsulated powders.

The empirical formula of lycopene is $C_{40}H_{56}$. Its molecular weight is 536.88.

9b. Chemical mode of action

(a) **Chemical interactions**: Lycopene can interact with other fat-soluble carotenoids, such as beta-carotene and lutein, to enhance the oxidative stability of edible oils.

(b) **Toxicity and environmental persistence**: Lycopene, as such or as a natural component of tomato products (juice, pulp, skins) and watermelon (pulp) is compostable and does not persist in the environment.

(c) **Environmental impacts from its use and/or manufacture**: Synthetic lycopene is produced in Ludwigshafen, Germany, according to an ISO 14000 Environmental Management System. The ISO 14000 Environmental Management System certificate for Ludwigshafen is available in Appendix D.

[6] http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153934.htm. Accessed May 24, 2011.

The production process conforms to the strict environmental requirements of Germany and the European Union. The manufacturer has analyzed the process to minimize or eliminate to the degree possible noxious and environment-unfriendly solvents and intermediates.

(d) **Effects on human health**: Lycopene has two well-established physiological effects. The obvious but harmless cosmetic effect of excessive intake of food or dietary supplements high in lycopene is the condition of "lycopenodermia," a reversible condition associated with a deep orange discoloration of the skin. This is not a worry in this application, since the amounts of lycopene used in infant formula are in the micro range. The other known health association of lycopene relates to prostate health in men. Dietary consumption of the carotenoid lycopene (mostly from tomato products) has been associated with a lower risk of prostate cancer. The remarkable chemopreventive and anti-proliferative activity of the antioxidant lycopene are related to its reactive oxygen species (ROS) scavenging activity, which allows lycopene to prevent lipid peroxidation and DNA damage.

(e) **Effects on soil organisms, crops, or livestock**: The largest organic cherry tomato producer in Europe apparently turns all the residues from its large greenhouse tomato production into fully sanitized and premium quality compost.[7] Thus lycopene does not adversely affect soil organisms.

10. Safety information about the substance including a Material Safety Data Sheet (MSDS) and a substance report from the National Institute of Environmental Health Studies.

The MSDS and product specification of **LycoVit® Dispersion 10%**, the material used to add lycopene to the edible oils of infant formula products, are available in Appendix D. This material is a 10.5% dispersion of crystalline lycopene in sunflower oil

The letter of FDA (see Appendix A) includes the following toxicological information:

> Using its proposed use levels and data from the United States Department of Agriculture 1994-1996 Continuing Surveys of Food Intakes by Individuals and 1998 Supplemental Children's Survey, BASF estimates that the intake of synthetic lycopene would be approximately 5 milligrams per person per day (mg/person/day) at the mean and approximately 11 mg/person/day at the 90th percentile. BASF notes that this estimate is comparable to the intake of lycopene from plant sources reported in the Third National Health and Nutrition Examination Survey and by the United States Department of Health and Human Services, National Center for Health Statistics, and Nutrition Coordinating Center.
>
> BASF discusses published and unpublished studies regarding the potential toxicity of synthetic and natural lycopene. BASF describes results from a published 13-week oral toxicity study conducted in rats fed BASF's commercial synthetic lycopene products. BASF concludes that results of the study support a no-observed-adverse-effect-level

[7] http://www.comp-any.com/company/index.php?id=21 Accessed June 1, 2011.

(NOAEL) for synthetic lycopene of 324 milligrams per kilogram body weight per day (mg/kg bw/day). BASF notes that this amount is approximately 4000-fold higher on a body weight basis than the mean estimated dietary intake (EDI) of synthetic lycopene. BASF also states that no adverse effects were reported in an unpublished developmental toxicity study conducted in rats and rabbits fed BASF's commercial synthetic lycopene products, and that no mutagenic effects were observed in unpublished genotoxicity studies conducted with BASF's commercial synthetic lycopene products. In addition, BASF notes that no adverse effects were observed in several published clinical studies conducted with either BASF's commercial synthetic lycopene products or natural lycopene.

The Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies of the European Food Safety Authority (EFSA) on the safety of synthetic lycopene, which was published in the EFSA Journal in 2008 and includes review of the toxicological testing of synthetic lycopene, is available as Appendix E. The EFSA report refers to a 20% dispersion of synthetic lycopene in edible oil. The lycopene material used to fortify infant formula is a more dilute 10% dispersion of crystalline lycopene in sunflower oil.

There is no substance report from NIEHS. However, the NIEHS website refers to lycopene as a protective substance. For example:

> Reduction of spontaneous mutagenesis in mismatch repair-deficient and proficient cells by dietary antioxidants. Mure, K, Rossman, T G. Published In *Mutat Res*, (2001 Sep 1)
>
> Abstract: Cells lacking mismatch repair (MMR) exhibit elevated levels of spontaneous mutagenesis. Evidence exists that MMR is involved in repair of some DNA lesions besides mismatches. If some oxidative DNA lesions are substrates for MMR, then the excess mutagenesis in MMR(-) cells might be blocked by dietary antioxidants. Effects of the dietary antioxidants ascorbate, alpha-tocopherol, (-)-epigallocatechin gallate (EGCG) and **lycopene** on spontaneous mutagenesis were studied using mismatch repair-deficient (hMLH1(-)) human colon carcinoma HCT116 cells and HCT116/ch3 cells, in which normal human chromosome 3 has been added to restore mismatch repair. HCT116 cells have a 22-fold higher spontaneous mutation rate compared with HCT116/ch3 cells. HCT116 cells cultured in 1% fetal bovine serum (FBS) have twice the spontaneous mutation rate of those cultured in 10% FBS, most likely due to reduction in serum antioxidants in the low serum medium. As expected, alpha-tocopherol (50 microM) and ascorbate (284 microM) reduced spontaneous mutagenesis in HCT116 cells growing in 1% serum more dramatically than in cells cultured in 10% serum. **The strongest antimutagenic compound was lycopene (5 microM)**, which reduced spontaneous mutagenesis equally (about 70%) in HCT116 cells growing in 10 and 1% FBS and in HCT116/ch3 cells. **Since lycopene was equally antimutagenic in cells growing in low and high serum, it may have another antimutagenic mechanism in addition to its antioxidant effect.** Surprisingly, EGCG (10 microM) was toxic to cells growing in low serum. It also reduced spontaneous mutagenesis equally (nearly 40%) in HCT116 and

> HCT116/ch3 cells. The large proportion of spontaneous mutagenesis that can be blocked by antioxidants in mismatch repair-deficient cells support the hypothesis that a major cause of their excess mutagenesis is endogenous oxidants. Blocking spontaneous mutagenesis, perhaps with a cocktail of antioxidants, should reduce the risk of cancer in people with a genetic defect in mismatch repair as well as other individuals.[8]

11. Research information about the substance which includes comprehensive substance research reviews and research bibliographies.

The following information is taken from a USDA Agricultural Research Service news release originating from the Grand Forks Human Nutrition Research Center:[9]

> Tomato's red hue comes mainly from a phytochemical called lycopene, a compound appearing to act as an anti-oxidant and neutralizing free radicals that damage cells of our body. Studies revealed that eating tomato may reduce the risk of cancer. In a study of over 40,000 health professionals, Harvard investigators reported that men who ate more than 10 servings of tomato-based foods daily could cut the risk of developing prostate cancer by 35 percent compared to those who ate the least amount of these foods.
>
> The benefits of lycopene were even more pronounced with advanced stages of prostate cancer. In another study of prostate cancer, researchers analyzed blood levels of lycopene and found that the risk of developing prostate cancer decreased with increased blood lycopene levels. The high level of lycopene in the blood was associated with low blood levels of prostate specific antigen, a biomarker of prostate health. Furthermore, studies indicated that tomato consumption may reduce the risk of colorectal, stomach and lung cancers.
>
> A greater intake of lycopene-rich tomato may benefit heart health by lowering the risk of cardiovascular disease. Studies showed that tomato lycopene prevented the oxidation of LDL "bad" cholesterol. In the process known as atherosclerosis, LDL cholesterol, fat and other substances are deposited in the arteries as plaque. When plaque builds up over time, it narrows the inner artery making it harder for blood to flow. Low levels of lycopene in blood are associated with increased mortality from heart disease (compared with higher levels). A clinical study showed that eight weeks of daily intake of a tomato extract was related to a significant drop in both systolic and diastolic blood pressure in patients with mild to moderate hypertension. The investigators assumed that this antihypertensive effect is a result of the antioxidative activity of the tomato extract.

A comprehensive review entitled “Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans,” was recently published in 2009 in the journal Molecular Nutrition and Food Research[10]. This review is available in Appendix F.

---

[8] http://tools.niehs.nih.gov/portfolio/index.cfm?action=portfolio.publicationdetail&id=323863. Accessed June 1, 2011.
[9] http://www.ars.usda.gov/News/docs.htm?docid=19428 . Accessed June 1, 2011.
[10] Mol. Nutr. Food Res. 2009, 53, S194 –S218.

12. A ''Petition Justification Statement'' which provides justification for inclusion of lycopene on the National List, §205.605(b)

Human colostrum and human milk contain carotenoids, including lycopene. Sommerburg et al.[11] reported that lycopene levels in colostrum – 121 mcg/L – were four times higher than in mature human milk – 32 mcg/L. Patton et al.[12] found that human colostrum contained 960 mcg/L of lycopene. Several researchers have reported on the lycopene content of mature human milk. Reported values vary from 10 mcg/L[13] to 17 mcg/L[14], 26 mcg/L[15], 27mcg/L[16], and as high as 93 mcg/L[17]. Including tomato sauce, a very bioavailable food source of lycopene, in the lactating woman's diet increased the lycopene content of her milk by 24 mcg/L within 3 days[12]. Term infants are born with measurable plasma lycopene levels that further increase when they are fed human milk after birth[13].

Unless an infant formula is deliberately fortified with lycopene, it will contain virtually none of this carotenoid. Sommerburg et al.[18] analyzed eight infant formula preparations and found no measurable lycopene in any of them. They observed that plasma lycopene levels in infants fed these infant formulas declined to low and even non-detectable levels within two weeks after birth.

The amount of lycopene deliberately added to the infant formulas described in Appendix C is in the range found in human milk.

Lycopene, the major pigment of tomato, can be extracted from tomato skins and other natural products. In fact, the Food and Drug Administration has issued several findings of no objections to self-affirmed GRAS status for tomato lycopene extract 6 percent, tomato lycopene extract 1.5 percent, and crystallized tomato lycopene extract (GRN No. 156), tomato pulp powder (GRN No. 163), concentrated tomato lycopene extract (GRN No. 185), palm-derived carotenoids with

---

[11] Carotenoid supply in breast-fed and formula-fed neonates. Sommerburg O, Meissner K, Nelle M, Lenhartz H, Leichsenring M. Eur J Pediatr. 2000 Jan-Feb;159(1-2):86-90.
[12] Patton S, Canfield LM, Huston GE, Ferris AM, Jensen RG. 1990. Carotenoids of human colostrum. Lipids 25:159–165.
[13] Canfield LM, Giuliano AR, Neilson EM, Yap HH, Graver EJ, Cui HA, Blashill BM. 1997. Beta-carotene in breast milk and serum is increased after a single beta carotene dose. *Am J Clin Nutr* 66:52–61.
[14] Giuliano AR, Neilson SM, Yap H-H, Baier M, Canfield LM. 1994. Quantitation of and inter/intra-individual variability in major carotenoids of mature human milk. *J Nutr Biochem* 5:551–556.
[15] Canfield LM, Giuliano AR, Neilson EM, Blashil BM, Graver EJ, Yap HH. 1998. Kinetics of the response of milk and serum beta-carotene to daily beta-car tene supplementation in healthy, lactating women. *Am J Clin Nutr* 67:276–283.
[16] Giuliano AR, Neilson EM, Kelly BE, Canfield LM. 1992. Simultaneous quantitation and separation of carotenoids and retinol in human milk by high-performance liquid chromatography. *Methods Enzymol* 213:391–399.
[17] Allen, CM, Smith, AM, Clinton, SK, Schwartz, SJ. 2002. Tomato consumption increases lycopene isomer concentrations in breast milk and plasma of lactating women. J Am Diet Assoc **102**(9): 1257-1262.
[18] Sommerburg O, Meissner K, Nelle M, Lenhartz H, Leichsenring M. Carotenoid supply in breast-fed and formula-fed neonates. 2000. Eur J Pediatr.159(1-2):86-90.

β-carotene, α-carotene, γ-carotene, and lycopene (GRN No. 320) and freeze-dried fruit gac powder (GRN No. 346). The problem with these natural materials is that they are not free of the proteins (allergens) of the plant from which they originate. Tomato particularly is a common allergen. To quote Speer[19]:

> The tomato is both a common food and a common allergen. It is an especially important cause of urticaria and eczema. Raw tomatoes are a common cause of apthae (canker sores) and contact dermatitis of the hand.

It is advisable to delay the introduction of common allergens until an infant is six months of age (or even older for infants of allergy-prone parents).

13. Confidential Business Information Statement

This petition contains no confidential business information.

[19] Food Allergy, 2nd Edition, Frederic Speer. John Wright-PSG Inc, Boston, 1983. p141.

Appendices

Petition for addition to the National List of the Synthetic Crystalline LYCOPENE on the National List of Substances Allowed as Ingredients in or on Processed Products Labeled as "organic" or "made with organic (specified ingredients or food group(s))."

Appendix A

- FDA Response Letter to GRAS Notice No. GRN 000119
- U.S. Patent No. 5,166,445

Appendix B – U.S. Regulations and International Standards

- Joint FAO/WHO Expert Committee on Food Additives (JECFA) Monograph
- FCC Specifications

Appendix C –Infant Formula Product Labels

Appendix D –

- MSDS and product specification of LycoVit® Dispersion 10%
- ISO 14000 Environmental Management System certificate for BASF Ludwigshafen

Appendix E – Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies of the European Food Safety Authority (EFSA)

Appendix F – Scientific review of carotenoids

U.S. Food and Drug Administration

Food

Agency Response Letter GRAS Notice No. GRN 000119

CFSAN/Office of Food Additive Safety
April 7, 2005

Herbert D. Woolf, Ph.D.
Technical Manager
BASF Corporation
3000 Continental Drive North
Mount Olive, NJ 07828-1234

Re: GRAS Notice No. GRN 000119

Dear Dr. Woolf:

This letter corrects an inadvertent error in the letter issued to you on May 22, 2003, in response to GRAS Notice No. 000119. In that letter, at the end of the second paragraph (now the third paragraph in this letter), the levels of use were incorrectly expressed as "0.5 to 7.0 percent."

The Food and Drug Administration (FDA) is responding to the notice, dated November 13, 2002, that you submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on November 20, 2003, filed it on November 27, 2003, and designated it as GRAS Notice No. GRN 000119.

The subject of the notice is synthetic lycopene. The notice informs FDA of the view of BASF Corporation (BASF) that synthetic lycopene is GRAS, through scientific procedures, for use as a direct food ingredient in breakfast cereals (ready-to-eat and cooked), drinks (juice drinks, energy drinks, and dairy fruit drinks), instant soup, low fat dressings, meal replacements, meatless meat products, nutrient bars, salty snacks, crackers and yogurt at levels ranging from 5 to 70 milligrams per kilogram of food.

For clarity, in this letter FDA uses the terms "lycopene," "synthetic lycopene," and "natural lycopene" as follows:

- We use the term "lycopene" to denote the chemical entity identified as Chemical Abstracts Service Registry Number 502-65-8. We use this term when describing the inherent properties of lycopene, regardless of the source of the lycopene.
- We use the term "synthetic lycopene" to denote the crystalline lycopene that is the subject of the notice and is produced by chemical synthesis.
- We use the term "natural lycopene" to denote the pigment that is produced during biosynthetic processes in developing plant tissue, such as that of tomato.

As part of its notice, BASF includes the report of a panel of individuals (BASF's GRAS panel) who evaluated the data and information that are the basis for BASF's GRAS determination. BASF considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to food. BASF's GRAS panel evaluated estimates of dietary exposure, method of production, and product specifications as well as published and unpublished studies. Based on this review, BASF's GRAS panel concluded that synthetic lycopene that meets its established food grade specifications is GRAS under the conditions of its intended use.

BASF describes generally available information about lycopene. Lycopene is an aliphatic hydrocarbon containing thirteen double bonds. Its molecular formula is $C_{40}H_{56}$. It is a member of the carotenoid class of compounds and is the most abundant carotenoid in ripe tomatoes. In tomatoes, as well as other fruits and vegetables, the all-*trans* isomer of lycopene predominates. However, storage, cooking, processing and exposure to light results in isomerization of the all-*trans* form to various *cis* forms.

BASF describes synthetic lycopene as a crystalline material derived from chemical synthesis and describes a three-stage process for this chemical synthesis.[(1)] Stage one produces an organic solution of C_{15} phosphonium methanesulfonate in dichloromethane (DCM), and stage two produces an organic solution of C_{10} dialdehyde in toluene. In stage three, the intermediates produced in stages one and two are gradually combined with sodium methoxide solution and undergo a condensation reaction to form crude lycopene. Glacial acetic acid and deionized water are added, the mixture is stirred vigorously, the aqueous and organic phases are allowed to separate, and the organic phase containing DCM and crude lycopene is extracted with water. Methanol is added to the organic phase, DCM is removed via distillation under reduced pressure, the crude methanolic lycopene solution is heated and then cooled to a crystalline slurry that is filtered and washed with methanol, and the lycopene crystals are then recrystalized and

dried under heated nitrogen. BASF notes that synthetic lycopene is stored under nitrogen or suspended in an aqueous solution containing antioxidants to prevent oxidation and isomerization of lycopene.

BASF prepares three commercial products from synthetic lycopene: Lycopene 10 Percent (tablet grade), Lycopene 10 Cold Water Dispersion (CWD), and Lycopene Dispersion 20 Percent (lycopene in vegetable oil). Lycopene 10 Percent (tablet grade) is a powder consisting of spherical particles of synthetic lycopene in a food starch coated matrix of gelatin and sucrose. The powder is stabilized with sodium ascorbate and ascorbyl palmitate and contains tricalcium phosphate as a flow-aid. Lycopene 10 CWD is a powder consisting of pulverized synthetic lycopene imbedded in a matrix of gelatin and glucose and stabilized with dl-alpha-tocopherol, ascorbyl palmitate and ascorbic acid. Lycopene Dispersion 20 Percent is a liquid that contains pulverized synthetic lycopene dispersed in oil. BASF notes that the three commercial lycopene products may be used in any of the food products described in its notice, although each commercial lycopene product has a distinctive formulation characteristic that would be more suitable for certain food products. For example, BASF states that Lycopene Dispersion 20 Percent is more suitable for food products in which there is an oil/fat phase.

BASF provides product specifications for synthetic lycopene. These specifications include limits on total carotenoids, arsenic, lead, copper, zinc, and heavy metals, loss on drying and residue on ignition. BASF also provides specification for the concentration of total carotenoids in the three commercial products. BASF provides typical values for percentages of *cis* and *trans* lycopene, but does not set specifications for these isomers. BASF reports that synthetic lycopene contains a minimum of 96 percent lycopene, although typical batches contain approximately 98 percent lycopene. BASF reports that the majority of lycopene in synthetic lycopene is in the form of *trans* isomers (70 to 84 percent) and notes that the *trans* isomer content reported for natural extracts of lycopene ranges from 67 to 98 percent. BASF also reports that some *cis* isomers of lycopene are also present in synthetic lycopene. BASF discusses lycopene-related substances and process residuals present in synthetic lycopene and identifies which substances are also present in extracts of tomatoes.

Using its proposed use levels and data from the United States Department of Agriculture 1994-1996 Continuing Surveys of Food Intakes by Individuals and 1998 Supplemental Children's Survey, BASF estimates that the intake of synthetic lycopene would be approximately 5 milligrams per person per day (mg/person/day) at the mean and approximately 11 mg/person/day at the 90th percentile. BASF notes that this estimate is comparable to the intake of lycopene from plant sources reported in the Third National Health and Nutrition Examination Survey and by the United States Department of Health and Human Services, National Center for Health Statistics, and Nutrition Coordinating Center.

BASF discusses published and unpublished studies regarding the potential toxicity of synthetic and natural lycopene. BASF describes results from a published 13-week oral toxicity study conducted in rats fed BASF's commercial synthetic lycopene products. BASF concludes that results of the study support a no-observed-adverse-effect-level (NOAEL) for synthetic lycopene of 324 milligrams per kilogram body weight per day (mg/kg bw/day). BASF notes that this amount is approximately 4000-fold higher on a body weight basis than the mean estimated dietary intake (EDI) of synthetic lycopene. BASF also states that no adverse effects were reported in an unpublished[(2)] developmental toxicity study conducted in rats and rabbits fed BASF's commercial synthetic lycopene products, and that no mutagenic effects were observed in unpublished genotoxicity studies conducted with BASF's commercial synthetic lycopene products. In addition, BASF notes that no adverse effects were observed in several published clinical studies conducted with either BASF's commercial synthetic lycopene products or natural lycopene.

BASF discusses published human studies related to the bioavailability of natural lycopene and BASF's commercial synthetic lycopene products. BASF reports that results of these studies showed no adverse effects on the absorption of other carotenoids. BASF notes, however, that excessive intake of food or dietary supplements high in carotenoids or lycopene has been associated with carotenodermia[(3)] and lycopenodermia[(4)], respectively.

Potential requirement for a color additive petition

In its notice, BASF notes that synthetic lycopene imparts color to food. As such, the use of synthetic lycopene in food products may constitute the use of a color additive under section 201(t)(1) of the Federal Food, Drug and Cosmetic Act (FFDCA) and FDA's implementing regulations in 21 CFR Part 70. Under section 201(t)(1) and 21 CFR 70.3(f), the term color additive means a material that is a dye, pigment, or other substance made by a process of synthesis or similar artifice, or extracted, isolated, or otherwise derived from a vegetable, animal, mineral, or other source, and that is capable (alone or through reaction with another substance) of imparting color when added or applied to a food except that such term does not include any material which the Secretary,[(5)] by regulation, determines is used (or intended to be used) solely for a purpose or purposes other than coloring. Under 21 CFR 70.3(g), a material that otherwise meets the definition of color additive can be exempt from that definition on the basis that it is used or intended to be used solely for a purpose or purposes other than coloring, as long as the material is used in a way that any color imparted is clearly unimportant insofar as the appearance, value, marketability, or consumer acceptability is concerned. Given the construct of section 201(t)(1) of the FFDCA and 21 CFR 70.3(f) and (g), the use of a substance that is capable of imparting color may constitute use as a color additive in addition to use as a food additive or GRAS substance. For example, beta-carotene is both approved for use as a color additive (21 CFR 73.95) and affirmed as GRAS for use as a nutrient supplement (21 CFR 184.1245); in some food products, beta-carotene is used for both purposes. Importantly, if the use of synthetic lycopene constitutes use as a color additive within the meaning of section 201(t)(1) of the FFDCA and FDA's implementing regulations in 21 CFR 70.3(f) and (g), section 721(a) of the

FFDCA requires premarket review and approval of that use by FDA. Under section 402(c) of the FFDCA, a food product that contains an unapproved color additive would be deemed adulterated.[6]

In its notice, BASF acknowledges that it intends to submit a color additive petition for uses of synthetic lycopene that would constitute use as a color additive, but is not explicit about its view on whether any of the uses already described in its GRAS notice would constitute use as a color additive. In a telephone conversation on February 25, 2003, between FDA and BASF, FDA requested that BASF present its view on this issue. In an amendment received by FDA on March 10, 2003, BASF presents its reasons for concluding that all of the intended uses of synthetic lycopene would be exempt from the definition of color additive under section 201(t) of the FFDCA and FDA's implementing regulations in 21 CFR 70.3(f) and (g). Importantly, FDA's response to GRN 000119 does not include any comment by FDA about BASF's view on this issue. If, after receipt of this letter, BASF has any specific questions about this issue, we recommend that you contact the Office of Food Additive Safety (OFAS), Division of Petition Review (HFS-265), 5100 Paint Branch Parkway, College Park, MD 20740. You can also reach this division by telephone at (202)418-3035

Potential labeling issues

Under section 403(a) of the Federal Food, Drug, and Cosmetic Act (FFDCA), a food is misbranded if its labeling is false or misleading in any particular. Section 403(r) of the FFDCA lays out the statutory framework for a health claim. In describing the intended use of synthetic lycopene and in describing the information that BASF relies on to conclude that synthetic lycopene is GRAS under the conditions of its intended use, BASF raises potential labeling issues under these provisions of the FFDCA. These labeling issues consist of BASF's assertion that synthetic lycopene has physiological effects that BASF views as beneficial. If products that contain synthetic lycopene bear any claims about such benefits on the label or in labeling, such claims are the purview of the Office of Nutritional Products, Labeling, and Dietary Supplements (ONPLDS) in the Center for Food Safety and Applied Nutrition (CFSAN). OFAS neither consulted with ONPLDS on these labeling issues nor evaluated the information in BASF's notice to determine whether it would support any claims made about synthetic lycopene on the label or in labeling.

Standards of Identity

In its notice, BASF states its intention to use synthetic lycopene in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity. If you have any questions about the use of synthetic lycopene in standardized foods that would be marketed in the United States, you should contact the staff in ONPLDS, Division of Food Labeling and Standards, 5100 Paint Branch Parkway, College Park, MD 20740. You can also reach this division by telephone at (301)436-2375.

Conclusions

Based on the information provided by BASF, as well as other information available to FDA, the agency has no questions at this time regarding BASF's conclusion that synthetic lycopene is GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of synthetic lycopene. As always, it is the continuing responsibility of BASF to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements. In particular, we note that any use of synthetic lycopene that constitutes use as a color additive requires premarket review and approval by FDA.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter, as well as a copy of the information in your notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at http://www.cfsan.fda.gov/~lrd/foodadd.html).

Sincerely,
Laura M. Tarantino, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

[1]FDA notes that steps in the three stage process for the synthesis of synthetic lycopene have been described in U.S. patents (issued to BASF and other companies) and journal articles. FDA notes that the Wittig reaction scheme for carotenoid synthesis is generally known and has been described in many books.

[2]Although BASF does not describe this study as a published study, an article that describes this study is currently in press and available on the Internet.

[3]A reversible condition associated with a yellowish discoloration of the skin.

[4]A reversible condition associated with a deep orange discoloration of the skin.

[5]The Secretary of the Department of Health and Human Services (DHSS). The Secretary of DHSS has delegated the authority for this provision of the FFDCA to FDA.

[6]We note that section 721(b)(4) of the FFDCA provides that a color additive shall be deemed to be safe and suitable for the purpose of listing under section 721(b) of the FFDCA while there is in effect a published finding of the Secretary declaring that the substance is exempt from the definition of "food additive" because of its being generally recognized by qualified experts as safe for its intended use as provided in section 201(s) of the FFDCA. Importantly, FDA's response to GRN 000119 does not constitute a "finding of the Secretary" within the meaning of section 721(b)(4) of the FFDCA.

Links on this page:

US005166445A

United States Patent [19]

Meyer

[11] Patent Number: 5,166,445

[45] Date of Patent: Nov. 24, 1992

[54] **METHOD FOR THE MANUFACTURE OF CAROTINOIDS AND THE NOVEL INTERMEDIATES**

[75] Inventor: **Karl Meyer**, Liestal, Switzerland

[73] Assignee: **Hoffman-La Roche Inc.**, Nutley, N.J.

[21] Appl. No.: **463,698**

[22] Filed: **Jan. 11, 1990**

[30] **Foreign Application Priority Data**

Feb. 10, 1989 [CH] Switzerland 468/89

[51] **Int. Cl.**[5] **C07F 5/02**

[52] **U.S. Cl.** **568/2**; 562/606; 562/607; 568/9

[58] **Field of Search** 562/606, 607; 568/9, 568/2; 558/286

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,755,457 8/1973 Carlson 562/607
4,182,731 1/1980 Schulz et al. 568/9

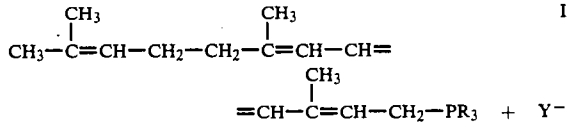
OTHER PUBLICATIONS

Manchand et al, *J. Chem. Soc.*, 2019–26 (1965).
Eidem et al., Acta Chemica Scandinavica B., 29, 1015–23 (1975).
Marki-Fischer et al., Helvetica Chimica Acta, 66 (Mar. 1983).
Chem. Abstracts. vol. 81 49884c. (1974).
Chem. Abstracts, vol. 84 44452n (1976).

Primary Examiner—Jose G. Dees
Assistant Examiner—B. Frazier
Attorney, Agent, or Firm—George M. Gould; William H. Epstein; Bruce A. Pokras

[57] **ABSTRACT**

Phosphonium salts of the formula



I

wherein A signifies aryl and Y^- signifies C_1–C_6-alkanoate or hydroxytrifluoroborate, as well as their manufacture from 3,7,11-trimethyldodeca-1,4,6,10-tetraen-3-ol with a triarylphosphine and with a C_1–C_6-alkanoic acid or a boron trifluoride etherate and, if desired after conversion into the phosphonium salt of a strong acid, further reaction to give lycopene.

1 Claim, No Drawings

METHOD FOR THE MANUFACTURE OF CAROTINOIDS AND THE NOVEL INTERMEDIATES

BACKGROUND OF THE INVENTION

The present invention is concerned with the manufacture of 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-phosphonium salts and of lycopene and with novel 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-phosphonium salts in this process.

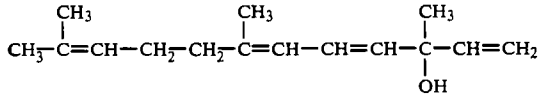
Lycopene is a natural red carotinoid which occurs e.g. in tomatoes.

Carotinoids are manufactured mainly by a Wittig reaction from phosphonium salts and aldehydes. The phosphonium salts which are used are salts of strong acids-usually halides, sulphates or phosphates-and are obtained as a rule from the corresponding alcohols, halides etc. However, the manufacture of lycopene according to this method gives only low yields, with especially in the production of the phosphonium salts predominantly unreactive byproducts being formed.

It has now been found that 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-phosphonium alkanoates and -phosphonium hydroxytrifluoroborates can be manufactured in high yield and that these can be reacted further in a Wittig reaction just as the usual phosphonium salts of strong acids. If desired, the alkanoates and hydroxytrifluoroborates can be converted, prior to the conversion into lycopene, in a simple manner into the phosphonium salts of usual strong acids, whereby the latter can be obtained via the alkanoates and hydroxytrifluoroborates in substantially higher yield.

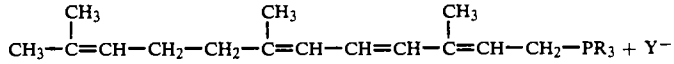
SUMMARY OF THE INVENTION

The invention is therefore concerned with a process for the manufacture of lycopene and intermediary phosphonium salts, which process comprises reacting 3,7,11-trimethyldodeca-1,4,6,10-tetraen-3-ol of the formula



II

with a triarylphosphine and with a C_1–C_6-alkanoic acid or a boron trifluoride etherate to give the phosphonium salt of the general formula



I

wherein R signifies aryl and Y^- signifies C_1–C_6-alkanoate or hydroxytrifluoroborate, if desired converting the phosphonium salt of formula I into the phosphonium salt of a strong acid and, if desired, reacting the phosphonium salt obtained with 2,7-dimethyl-2,4,6-octatrienedial to give lycopene.

The invention is also concerned with the novel phosphonium salts of formula I.

DETAILED DESCRIPTION

The term "aryl" above denotes usual aryl residues which are present in phosphines and phosphonium salts, such as phenyl, tolyl, naphthyl and the like, especially phenyl.

The term "C_1–C_6-alkanoate" denotes the anion of straight-chain or branched C_1–C_6-alkanoic acids such as formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid and the like. The acetate and formate are preferred alkanoates. Accordingly, formic acid and acetic acid are preferred alkanoic acids.

The term "hydroxytrifluoroborate" denotes the anion $B(OH)F_3^-$.

The term "boron trifluoride etherate" embraces usual etherates of boron trifluoride, especially the etherates of straight-chain, branched or cyclic ethers having up to 8 carbon atoms such as dimethyl ether, diethyl ether, diisopropyl ether, t-butyl methyl ether, di-t-butyl ether, tetrahydrofuran and the like. Boron trifluoride diethyl etherate is especially preferred.

The term "strong acid" embraces hydrohalic acids (especially hydrochloric acid and hydrobromic acid), sulphuric acid, sulphonic acids (such as benzenesulphonic acid and toluenesulphonic acid), phosphoric acid and other inorganic or organic acids having a comparable degree of dissociation. The term "phosphonium salt of a strong acid" therefore denotes the phosphonium halides (especially the chlorides and bromides), sulphates, hydrogen sulphates, sulphonates, phosphates and the like.

The term "halogen" denotes in the scope of the present invention fluorine, chlorine, bromine and iodine, especially chlorine and bromine.

Formula I above embraces pure isomers and mixtures of isomers. Preferably, however, the 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl residue, which is depicted in formula I and which is referred to in the literature also as the Ψ-ionylidene-ethyl residue or pseudoionylidene-ethyl residue, is present in the all-E form. The phosphonium salts of formula I and the phosphonium salts of a strong acid which are obtainable therefrom are accordingly preferably 3,7,11-trimethyldodeca-2E,4E,6E,10-tetraen-1-yl-phosphonium salts (referred to hereinafter as the "all-E isomer"). Other preferred isomers are the 3,7,11-trimethyldodeca-2Z,4E,6E,10-tetraen-1-yl-phosphonium salts (referred to hereinafter as the "2Z isomer") and the 3,7,11-trimethyldodeca-2E,4E,6Z,10-tetraen-1-yl-phosphonium salts (referred to hereinafter as the "6Z isomer").

The 3,7,11-trimethyldodeca-1,4,6,10-tetraen-3-ol (also referred to as vinyl-Ψ-ionol or vinylpseudoionol) which is used as the educt in the reaction in accordance with the invention is known e.g. from Acta Chemica Scandinavica B29, 1015 (1975) and J. Chem. Soc. 1965, 2019 and can be obtained from pseudoionone according to known methods, e.g. by reaction with a vinyl-Grignard reagent or by reaction with lithium and acetylene and subsequent partial hydrogenation of the triple bond in the presence of a Lindlar catalyst. 3,7,11-Trimethyldodeca-1,4,6,10-tetraen-3-ol can be used as a mixture of isomers or as a pure isomer. Mixtures of isomers can be separated, if desired, by fractional distillation.

The reaction of 3,7,11-trimethyldodeca-1,4,6,10-tetraen-3-ol with a triarylphosphine and with a C_1–C_6-alkanoic acid or a boron trifluoride etherate can be effected in a manner known per se. The triarylphosphine is preferably used in at least about equimolar

amounts, for example about 1-3 equivalents. In general, it is especially preferred to use a small excess of triarylphosphine, e.g. about 1.1-2 equivalents. Triphenylphosphine is the preferred triarylphosphine. The alkanoic acid and, respectively, the boron trifluoride etherate are conveniently used in at least about equimolar amounts. As a rule, a clear excess is preferred. Therefore, there are in general preferably used at least about 3 equivalents, particularly at least about 8 equivalents, of alkanoic acid and, respectively, boron trifluoride etherate. Formic acid and acetic acid are the preferred alkanoic acids and boron trifluoride diethyl etherate is the preferred boron trifluoride etherate. The reaction is conveniently effected in a solvent, whereby, however, the alkanoic acid or the ether corresponding to the etherate which is used can itself serve as the solvent and/or an inert organic solvent can be added to the mixture. Preferred inert organic solvents are optionally chlorinated or aromatic hydrocarbons, ethers, alcohols and esters such as hexane, methylene chloride, chloroform, benzene, toluene, xylene, diisopropyl ether, t-butyl methyl ether, tetrahydrofuran, methanol, ethanol, ethyl acetate and the like.

A preferred embodiment of the process in accordance with the invention comprises carrying out the reaction using formic acid in the presence of an inert organic solvent (especially in the presence of an optionally chlorinated or aromatic hydrocarbon). A further preferred embodiment comprises carrying out the reaction using acetic acid (or generally using a C_2-C_6-alkanoic acid) with or without the addition of an inert organic solvent (for example an optionally chlorinated or aromatic hydrocarbon). A third preferred embodiment comprises carrying out the reaction using a boron trifluoride etherate in the presence of an inert organic solvent (preferably an optionally chlorinated or aromatic hydrocarbon or an ether).

Temperature and pressure are not critical in the reaction of 3,7,11-trimethyldodeca-1,4,6,10-tetraen-3-ol. In general, the reaction is carried out at about 0°-100° C. and under normal pressure. A temperature range of about 40°-80° C., especially about 50°-65° C., is preferred.

The phosphonium salt of formula I which is obtained can be isolated from the reaction mixture if desired. As these phosphonium salts are, however, less stable in isolated form, they are preferably without previous isolation converted directly into lycopene or converted into more stable phosphonium salts by anion exchange.

The anion exchange, i.e. the conversion of the phosphonium salts of formula I into corresponding phosphonium salts of a strong acid, can be effected by reaction with a strong acid or with a soluble salt of the strong acid. The reaction can be carried out preferably using an aqueous solution of the strong acid or of the salt of the strong acid. It can preferably be effected during the working-up of the reaction mixture containing the phosphonium salt of formula I, for example by washing with the aqueous solution of the acid or of the salt. The alkali metal salts, especially the sodium salts and the potassium salts, are the preferred soluble salts of the strong acids. Hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid and sulphonic acids such as benzenesulphonic acid and toluenesulphonic acid are preferred strong acids. The alkali metal salts of these acids such as sodium chloride, potassium chloride, sodium bromide, sodium sulphate, sodium hydrogen sulphate, sodium phosphate, sodium tosylate and the like are especially preferred salts. The conversion into the chloride is especially preferred; it can be effected preferably using aqueous hydrochloric acid or using an aqueous solution of an alkali metal chloride (e.g. sodium chloride).

The conversion into the phosphonium salt of a strong acid by anion exchange is preferred as a rule when a purification or separation of isomers is effected prior to the Wittig reaction. Alternatively, the phosphonium salt of formula I can preferably be converted directly into lycopene.

The reaction of the phosphonium salts of formula I or of the corresponding phosphonium salts of strong acids with 2,7-dimethyl-2,4,6-octatrienedial can be carried out in a manner known per se and under conditions which are usual for Wittig reactions. Suitable conditions are well-known to the person skilled in the art. Examples of preferred methods are the reaction in methylene chloride/methanol in the presence of sodium methylate, in toluene/methanol in the presence of sodium methylate, in ethanol in the presence of sodium ethylate or in methylene chloride/water in the presence of potassium hydroxide or potassium carbonate. The reaction is preferably effected at about 0°-50° C. 2,7-Dimethyl-2E,4E,6E-octatrienedial is preferably used for the manufacture of all-trans-lycopene. The double bonds which newly result in the Wittig reaction are formed partially in the cis form. They can, however, be isomerized to the trans form according to usual methods (e.g. thermally). The isomerization often takes place even under the reaction conditions.

In the conversion of 3,7,11-trimethyldodeca-1,4,6,10-tetraen-3-ol into the phosphonium salt of formula I the configuration of the double bond in position 6 of 3,7,11-trimethyldodeca-1,4,6,10-tetraen-3-ol is largely retained. The double bond in position 4 of formula I is as a rule obtained in the E form independently of the configuration in the educt. The double bond in position 2 of formula I is mainly formed predominantly in the E form with a small amount of Z form. In order to avoid isomerization reactions as far as possible, 3,7,11-trimethyldodeca-1,4E,6E,10-tetraen-3-ol or a mixture of isomers having a high content of this isomer is therefore preferably used for the manufacture of all-E-lycopene. 3,7,11-Trimethyldodeca-1,4Z,6E,10-tetraen-3-ol is, however, equally well suited.

The present invention is illustrated in more detail by the following Examples. The yield of phosphonium salts and the isomer ratios were determined by high-pressure liquid chromatography (HPLC) with an internal standard for reasons of stability. The structures given were confirmed by nuclear magnetic resonance spectroscopy.

EXAMPLE 1

A mixture of 52.46 g of triphenylphosphine and 525 ml of glacial acetic acid was heated to 55° C. and then treated dropwise within 10 minutes with a mixture of 25.81 g of 3,7,11-trimethyldodeca-1,4E,6E,10-tetraen-3-ol (purity 85.3%) and 15 ml of hexane. Thereby, the temperature rose to 60° C. The mixture was stirred at 60° C. for a further 2.5 hours and then cooled to 25° C.

Subsequently, the reaction mixture containing 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium acetate [Rf value 0.41 (butyl acetate/formic acid/water 88:10:2)] was rinsed with 500 ml of methylene chloride into a separating funnel and, for the conversion into the chloride, washed six times with 500 ml

of 2 percent sodium chloride solution each time. The organic phase was concentrated on a rotary evaporator. The residue was taken up in 200 ml of hexane and 300 ml of methanol/water (vol. 80:20). The hexane phase was separated and the methanol/water phase was extracted four times with 200 ml of hexane each time. The combined hexane phases were back-extracted twice with 20 ml of methanol/water (vol. 80:20) each time. The methanol/water phases were freed from methanol on a rotary evaporator at 40° C. The aqueous solution was rinsed with 200 ml of methylene chloride into a separating funnel and washed three times with 200 ml of 10 percent sodium chloride solution each time. The organic phase was concentrated on a rotary evaporator. In order to remove the methylene chloride completely, the residue was treated twice with ethyl acetate and concentrated each time. The crystalline crude product was taken up in ethyl acetate (a total of 420 g of mixture) and brought into solution by adding 4.47 ml of methanol and heating to 40° C. The solution was left to stand at room temperature overnight and was then filtered. The filter cake was washed with ethyl acetate.

The mother liquor containing the desired product was analyzed by HPLC with an internal standard. The analysis gave 36.67 g (73.2%) of 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium chloride consisting of 28.78 g (57.5%) of all-E isomer, 5.62 g (11.2%) of 2Z isomer and 2.27 g (4.5%) of 6Z isomer. Rf value 0.41 (butyl acetate/formic acid/water 88:10:2). The filter cake, which contained a further 7.19 g (14.3%) of product consisting of 2.13 g (4.2%) of all-E isomer, 1.48 g (3.0%) of 2Z isomer and 3.58 g (7.1%) of 6Z isomer, was not worked-up.

Analogous experiments (without crystallization) at 40° C., 60° C. and 80° C. yielded the following results in accordance with HPLC analysis:

Temperature	Reaction time	Chemical yield	Amount of 6Z isomer
40° C.	6.5 h	70%	8.6%
60° C.	2.5 h	96%	10.9%
80° C.	1.5 h	96%	11.7%

EXAMPLE 2

A mixture of 2.45 g of triphenylphosphine, 25 ml of methylene chloride and 4 ml of glacial acetic acid was heated to 40° C. and treated dropwise under argon within 10 minutes with 1.53 g of 3,7,11-trimethyldodeca-1,4E,6Z,10-tetraen-3-ol (purity 76.6%). The reaction mixture was stirred at 40° C. overnight and then concentrated on a rotary evaporator. The residue was transferred with 50 ml of methanol/water (vol. 80:20) into a separating funnel and extracted four times with 30 ml of hexane each time. The combined hexane phases were backwashed with 30 ml of methanol/water (vol. 80:20). The combined methanol phase containing the desired product was analyzed by HPLC with an internal standard. It contained 0.029 g (2.5%) of unreacted 3,7,11-trimethyldodeca-1,4,6,10-tetraen-3-ol and 2.26 g (81.3%) of 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium acetate consisting of 1.36 g (48.9%) of 6Z isomer, 0.64 g (23.0%) of all-E isomer and 0.26 g (9.4%) of 2Z isomer. Rf value 0.41 (butyl acetate/formic acid/water 88:10:2).

EXAMPLE 3

A mixture of 2.45 g of triphenylphosphine, 25 ml of hexane and 4 ml of glacial acetic acid was heated to 45° C. and treated dropwise under argon within 10 minutes with 1.53 g of 3,7,11-trimethyldodeca-1,4E,6Z,10-tetraen-3-ol (purity 76.6%). The reaction mixture was stirred at 45° C. overnight and then concentrated on a rotary evaporator. The residue was transferred with 50 ml of methanol/water (vol. 80:20) into a separating funnel and extracted four times with 30 ml of hexane each time. The combined hexane phases were back-washed with 30 ml of methanol/water (vol. 80:20). The combined methanol phase containing the desired product was analyzed by HPLC with an internal standard. It contained 0.03 g (3%) of unreacted 3,7,11-trimethyldodeca-1,4,6,10-tetraen-3-ol and 2.14 g (77.0%) of 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium acetate consisting of 1.22 g (43.9%) of 6Z isomer, 0.67 g (24.1%) of all-E isomer and 0.25 g (9.0%) of 2Z isomer. Rf value 0.41 (butyl acetate/formic acid/water 88:10:2).

EXAMPLE 4

A mixture of 7.85 g of triphenylphosphine, 78.5 ml of hexane and 9.5 ml of formic acid was treated dropwise while stirring and gassing with nitrogen at 60° C. within 10 minutes with a solution of 8.3 g of 3,7,11-trimethyldodeca-1,4E,6E,10-tetraen-3-ol (purity 66.5%) in 8 ml of hexane. The reaction mixture was stirred at 60° C. for a further 2.5 hours and then rinsed with 125 ml of methanol/water (vol. 80:20) into a separating funnel. The hexane phase was separated and the methanol phase was washed three times with 125 ml of hexane each time. The hexane phases were back-extracted twice with 25 ml of methanol/water (vol. 80:20) each time. The methanol phases containing 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium formate [Rf value 0.41 (butyl acetate/formic acid/water 88:10:2)] were combined and evaporated.

The residue was taken up in 200 ml of methylene chloride and, for the conversion into the chloride, washed five times with 250 ml of 2 percent sodium chloride solution each time. The organic phase was evaporated and the residue was taken up in methanol and analyzed by HPLC with an internal standard. The product solution contained 12.31 g (96.3%) of 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium chloride consisting of 7.97 g (62.3%) of all-E isomer, 2.8 g (21.9%) of 6Z isomer and 1.54 g (12.1%) of 2Z isomer. Rf value 0.41 (butyl acetate/formic acid/water 88:10:2).

EXAMPLE 5

A mixture of 3.2 g of triphenylphosphine, 11.9 ml of methylene chloride and 1.53 ml of formic acid was treated dropwise at 30° C. within 60 minutes with 1.35 g of 3,7,11-trimethyldodeca-1,4E,6E,10-tetraen-3-ol (purity 66.3%). The mixture was stirred at 30° C. for a further 17 hours.

Subsequently, the reaction mixture containing 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium formate [Rf value 0.41 (butyl acetate/formic acid/water 88:10:2)] was rinsed with 50 ml of methylene chloride into a separating funnel and, for the conversion into the chloride, washed twice with 125 ml of 2 percent sodium chloride solution each time. The organic phase was concentrated on a rotary evaporator and the resi-

due was taken up in methanol and analyzed by HPLC with an internal standard. The product solution contained 1.69 g (81.8%) of 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium chloride consisting of 1.14 g (55.1%) of all-E isomer, 0.32 g (15.6%) of ZZ isomer and 0.23 g (11.1%) of 6Z isomer. Rf value 0.41 (butyl acetate/formic acid/water 88:10:2).

EXAMPLE 6

A mixture of 2.27 g of triphenylphosphine, 22.5 ml of toluene and 0.85 ml of formic acid was treated dropwise at 55° C. within 5 minutes with 1.1 g of 3,7,11-trimethyldodeca-1,4E,6Z,10-tetraen-3-ol (purity about 75%). In so doing the temperature rose to 60° C. The mixture was stirred at 60° C. for a further 2.5 hours.

Subsequently, the reaction mixture containing 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium formate [Rf value 0.41 (butyl acetate/formic acid/water 88:10:2)] was rinsed with 50 ml of toluene into a separating funnel and, for the conversion into the chloride, washed twice with 125 ml of 2 percent sodium chloride solution each time. The organic phase was concentrated on a rotary evaporator and the residue was taken up in methanol and analyzed by HPLC with an internal standard. The product solution contained 1.618 g (84.2%) of 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium chloride consisting of 0.931 g (48.2%) of 6Z isomer, 0.480 g (25.2%) of all-E isomer and 0.207 g (10.8%) of 2Z isomer. Rf value 0.41 (butyl acetate/formic acid/water 88:10:2).

EXAMPLE 7

A mixture of 3.54 g of triphenylphosphine, 50 ml of t-butyl methyl ether and 3.32 g of 3,7,11-trimethyldodeca-1,4E,6E,10-tetraen-3-ol (purity 66.3%) was treated dropwise at 53° C. within 35 minutes with a solution of 1.30 ml of boron trifluoride diethyl etherate in 25 ml of t-butyl methyl ether. The mixture was stirred at 53° C. for a further 2.5 hours and then concentrated on a rotary evaporator. The residue was rinsed with 80 ml of methanol/water (vol. 80:20) into a separating funnel and extracted four times with 50 ml of hexane each time. The hexane phases were combined and back-washed twice with 10 ml of methanol/water (vol. 80:20) each time. The methanol phases containing 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium hydroxytrifluoroborate [Rf value 0.41 (butyl acetate/formic acid/water 88:10:2)] were concentrated on a rotary evaporator.

The residue was rinsed with 80 ml of methylene chloride into a separating funnel and, for the conversion into the chloride, washed three times with 125 ml of 2 percent sodium chloride solution each time. The organic phases were concentrated on a rotary evaporator and the residue was taken up in methanol and analyzed by HPLC with an internal standard. The product solution contained 3.404 g (61.9%) of 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium chloride consisting of 2.403 g (43.7%) of all-E isomer, 0.528 g (9.6%) of 2Z isomer and 0.473 g (8.6%) of 6Z isomer. Rf value 0.41 (butyl acetate/formic acid/water 88:10:2).

EXAMPLE 8

41.2 g of 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium chloride (prepared according to Example 1) and 5.4 g of 2,7-dimethyl-2E,4E,6E-octatrienedial were dissolved in 410 ml of methylene chloride. The solution was cooled to −5° C. and treated dropwise within 15 minutes with 25.1 g of sodium methylate solution (containing 4.7 g of sodium methylate in 20.4 g of methanol). The reaction mixture was stirred at −5° C. for 30 minutes, then warmed to 20° C. within 10 minutes and stirred at 20° C. for a further 60 minutes. Subsequently, the reaction mixture was transferred into a separating funnel and washed twice with 300 ml of 1 percent sodium chloride solution each time. The organic phase was concentrated to a volume of 100 ml on a rotary evaporator. Subsequently, the methylene chloride was evaporated under normal pressure and replaced by methanol. The mixture was left to cool and was stored at 5° C. overnight in order to complete the crystallization. The crystallizate was filtered off under suction and washed with 50 ml of methanol. There were thus obtained 18.2 g of lycopene in a purity of 86.1% (according to HPLC with an internal standard). The yield was 89.0% based on 2,7-dimethyl-2E,4E,6E-octatrienedial. The mother liquor, which contained a further 2% of lycopene, was not worked-up.

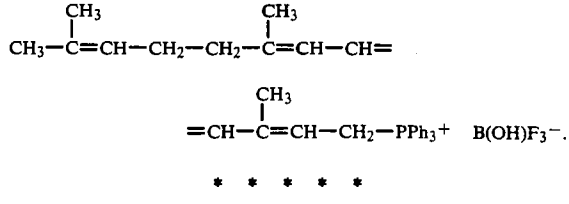
EXAMPLE 9

A mixture of 5.25 g of triphenylphosphine and 52.5 ml of glacial acetic acid was heated to 55° C. and then treated dropwise within 10 minutes with 2.34 g of 3,7,11-trimethyldodeca-1,4E,6E,10-tetraen-3-ol (purity 84.4%). The reaction mixture was stirred at 60° C. for a further 2.5 hours, then cooled to room temperature and concentrated on a rotary evaporator to a large extent. The residue, which still contained some glacial acetic acid, was rinsed with 100 ml of hexane and 100 ml of methanol/water (vol. 80:20) into a separating funnel and extracted three times with 100 ml of hexane each time. The combined hexane phases were back-washed twice with 10 ml of methanol/water (vol. 80:20) each time and then concentrated on a rotary evaporator. The residue was taken up immediately in 100 ml of methylene chloride. The methylene chloride phase was washed with water and concentrated on a rotary evaporator. There were thus obtained 4.0 g of a crude product of 3,7,11-trimethyldodeca-2,4,6,10-tetraen-1-yl-triphenylphosphonium acetate which was further reacted immediately.

The phosphonium acetate obtained and 0.5 g of 2,7-dimethyl-2E,4E,6E-octatrienedial were dissolved in 40 ml of methylene chloride. The solution was cooled to −10° C. and treated dropwise within 5 minutes with 2.4 g of sodium methylate solution (containing 0.44 g of sodium methylate in 1.96 g of methanol). The reaction mixture was stirred at −10° C. for 2.5 hours, then warmed to room temperature and treated with 70 ml of water. Subsequently, the reaction mixture was transferred with methylene chloride into a separating funnel and the aqueous phase was separated. The lycopene obtained was detected by thin-layer chromatography; Rf value 0.92 (methylene chloride/diethyl ether 99:1).

I claim:

1. A compound of the formula



* * * * *

LYCOPENE (SYNTHETIC)

New specifications prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006). A group ADI "not specified" for lycopene from all sources was established at the 71st JECFA (2009).

SYNONYMS

INS 160d(i)

DEFINITION

Synthetic lycopene is produced by the Wittig condensation of synthetic intermediates commonly used in the production of other carotenoids used in food. Synthetic lycopene consists predominantly of all-*trans*-lycopene together with 5-*cis*-lycopene and minor quantities of other isomers. Commercial lycopene preparations intended for use in food are formulated as suspensions in edible oils or water-dispersible powders and are stabilised with antioxidants.

Chemical names

Ψ,*Ψ*-carotene
all-*trans*-lycopene
(all-E)-lycopene
(all-E)-2,6,10,14,19,23,27,31-octamethyl-2,6,8,10,12,14,16,18,20,22,24,26,30-dotriacontatridecaene

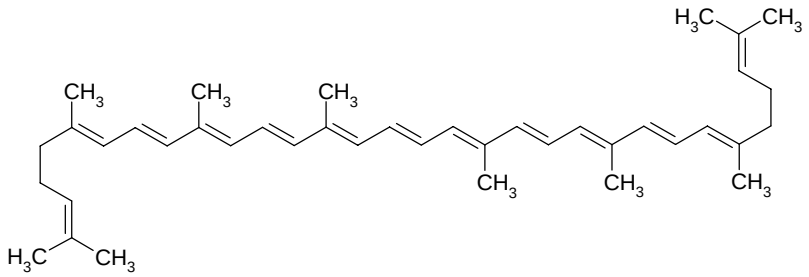
CAS number

502-65-8

Chemical formula

$C_{40}H_{56}$

Structural formula



Formula weight

536.9

Assay

Not less than 96% total lycopenes; not less than 70% all-*trans*-lycopene

DESCRIPTION

Red crystalline powder

FUNCTIONAL USES

Colour, nutrient supplement

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Insoluble in water, freely soluble in chloroform

Test for carotenoids

The colour of the solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrate and 1N sulfuric acid

Solution in chloroform — A 1% solution is clear and has intensive red-orange colour

Spectrophotometry (Vol. 4) — A solution in hexane shows an absorption maximum at approximately 470 nm

PURITY

Loss on drying (Vol. 4) — Not more than 0.5% (40°, 4 h at 10 mm Hg)

Lead (Vol. 4) — Not more than 1 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

Apo-12'-lycopenal — Not more than 0.15%
See description under TESTS

Triphenyl phosphine oxide (TPPO) (Vol. 4) — Not more than 0.01%

TESTS

PURITY TESTS

Apo-12'-lycopenal — Determine by HPLC using the following conditions:

Reagents (Note: all solvents should be HPLC-grade)
Hexane
Triethylamine (TEA)
Tetrahydrofuran (THF)
Toluene stabilised with BHT (0.5 g BHT in 1000 ml toluene)
Apo-12'-lycopenal (also known as lycopene C_{25}-aldehyde) standard (available from DSM Nutritional Products)

Apparatus
HPLC system with a suitable pump, injector, and integrator
Column: Stainless steel (200x4.0 mm)
Stationary phase: Nucleosil Si 100 3 µm (Macherey-Nagel or equivalent)
Detector: UV/VIS or VIS

HPLC conditions
Flow: 2.0 ml/min
Injection volume: 5.0 µl
Pressure: approx. 135 bar
Detection: 435 nm
Mobile phase: A – hexane
B – Hexane:TEA (99.9:0.1) (v/v)
C – Hexane:THF (80:20) (v/v)

Gradient

Time, min	A%	B%	C%
0	80	20	0
16	60	20	20
22	40	20	40
24.5	80	20	0

Run time
Approximately 25 min.

Standard solution
Accurately weigh between 14.5 and 15.5 mg of the *apo*-12'-lycopenal standard into a 50-ml volumetric flask. Dissolve in toluene stabilised with BHT and make up to volume. Transfer 2 ml of the solution into 100-ml volumetric flask and add toluene stabilised with BHT to volume.

Sample solution
Accurately weigh between 29.0 and 31.0 mg of the sample into a 10-ml volumetric flask and dissolve and dilute to volume with toluene stabilised with BHT. Put the solution in an ultrasonic bath for 10 min.

Results
The retention time of a*po*-12'-lycopenal is approximately 14 min. The relative retention time of *apo*-12'-lycopenal with respect to all-*trans*-lycopene is 1.6.

Calculation

$$Apo\text{-}12'\text{-lycopenal}\,(\%) = \frac{A_S \times W_{St} \times 10}{A_{St} \times W_S \times 2500} \times 100$$

where
A_S is the peak area of the sample;
A_{St} is the peak area of the standard;
W_{St} is the weight of the standard (mg);
W_S is the weight of the sample (mg);
10 is the volume of the volumetric flask in which the sample was dissolved (ml); and
2500 is the volume of the volumetric flask in which the standard was dissolved (50 ml) multiplied by dilution (50).

METHOD OF ASSAY

Determine total lycopenes and all-*trans*-lycopene by HPLC using the following conditions:

Reagents (Note: all solvents should be HPLC-grade)
Hexane
Tetrahydrofuran stabilised with 0.025% BHT
N-Ethyl-diisopropylamine
Lycopene standard (purity 95% or higher; available from CaroteNature GmbH)

Apparatus
Spectrophotometer with a 1-cm cuvette
HPLC system with a suitable pump, injector, thermostated column compartment, and integrator

Column:	Two serially-connected two stainless steel columns (250x4.0 mm)
Stationary phase:	Nucleosil 300-5, 5 µm (Macherey-Nagel or equivalent)
Detector:	UV/VIS or VIS

HPLC conditions

Flow rate: 0.8 ml/min
Injection volume: 20µl
Pressure: approx. 80 bar
Column temperature: 20°
Detection: 470 nm
Mobile phase: 0.15% solution of N-ethyl-diisopropylamine in hexane (v/v)
Run time: 30 min

HPLC standard solution

Accurately weigh between 5.5 and 6.5 mg of the lycopene standard into a 100-ml volumetric flask. Dissolve in 5 ml of tetrahydrofuran stabilised with BHT and make up to volume with hexane. This is a standard solution for the HPLC assay.

Spectrophotometric standard solution

Transfer 5.0 ml of the HPLC standard solution into a 100-ml volumetric flask and make up to volume with hexane. This is a standard solution for the spectrophotometric determination of lycopene in the lycopene standard.

Sample solution

Accurately weigh between 4.5 and 5.5 mg of the sample into a 100-ml volumetric flask. Dissolve in 5 ml of tetrahydrofuran stabilised with BHT and make up to volume with hexane.

Spectrophotometric determination of lycopene

Measure the absorbance of the spectrophotometric standard solution in a 1-cm cuvette at the wavelength of maximum absorption (approximately 470 nm). Use hexane as the blank.

Calculation

$$C_{St}\,(mg/l) = \frac{A \times 10000}{3450}$$

where

C_{St} is the lycopene concentration in the spectrophotometric standard solution (mg/l);

A is absorbance at the wavelength of maximum absorption;

3450 is the specific absorbance $A_{1cm}^{1\%}$ of all-*trans*-lycopene in hexane; and

10000 is the scaling factor.

HPLC analysis

Repeatedly inject 20 µl of the HPLC standard solution. Record the total peak area of all detected lycopene isomers (exclude the solvent peak). Calculate the mean peak area from repeated injections and calculate the lycopene response factor (RF) according to the formula:

$$RF = \frac{A_{St}}{C_{St} \times 20}$$

where
RF is the response factor of lycopene (AU x l/mg);
A_{St} is the mean peak area of all lycopene peaks (AU);
C_{St} is the concentration of lycopene in the spectrophotometric standard solution (mg/l); and
20 is the dilution factor used in the preparation of the spectrophotometric standard solution from the HPLC standard solution.

Inject the sample solution and record the peak areas of lycopene isomers.

Results
Retention times

Lycopene isomer	Relative retention time*	Absolute retention time (approx.)
13-*cis*-lycopene	0.6	14 min
9-*cis*-lycopene	0.8	19 min
All-*trans*-lycopene	1.0	22 min
5-*cis*-lycopene	1.1	24 min

* relative to all-*trans*-lycopene

Calculations

Calculate the content of total lycopenes according to the formula:

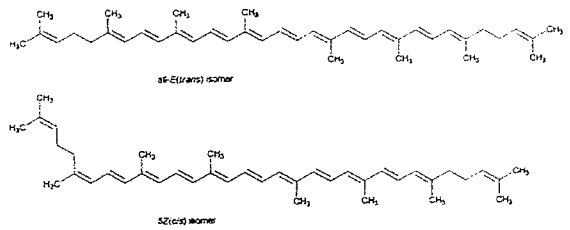
$$\text{Total lycopenes}\,(\%) = \frac{(A_{trans} + A_{5cis} + A_{9cis} + A_{13\,cis} + A_{xcis}) \times 0.1}{RF \times W_S} \times 100$$

Where:
A_{trans} is the peak area of all-*trans*-lycopene (AU);
A_{5cis}, A_{9cis}, and A_{13cis} are the peak areas of 5*cis*-, 9*cis*-, and 13*cis*-lycopene (AU);
A_{xcis} is the peak area of other *cis* isomers, if detected (AU);
0.1 is the volume of the flask in which the sample was dissolved (l);
RF is the response factor of lycopene (AU x l/mg); and
W_S is the weight of the sample (mg).

Calculate the content of all-*trans*-lycopene as follows:

$$\text{All - }trans\text{ - lycopene}\,(\%) = \frac{A_{trans} \times 0.1}{RF \times W_S} \times 100$$

Lycopene, Synthetic



$C_{40}H_{56}$ Formula wt 536.88

CAS: All-*E*(*trans*) lycopene [502-65-8]

CAS: 5*Z*(*cis*) lycopene [101468-86-4]

DESCRIPTION

Lycopene, Synthetic occurs as dark red to dark violet crystals or crystalline powder. It is an open-chain, unsaturated C_{40} carotenoid made up of eight isoprene units, and consists of a mixture of geometric isomers. It is insoluble in water and in acids and alkalis, and nearly insoluble in methanol and in ethanol, but it is soluble in chloroform and in tetrahydrofuran. It is sparingly soluble in ether, in hexane, and in vegetable oils.

Function Source of lycopene

Packaging and Storage Store under inert gas in tight, light-resistant containers in a cool place.

IDENTIFICATION

- **A. UV-Visible Absorption Spectrum**

  Standard stock solution: Transfer 25 mg of lycopene Reference Standard (Chromadex, Inc., Santa Ana, CA; Sigma, St. Louis, MO, or equivalent) into a 100-mL volumetric flask, add about 25 mg of butylated hydroxytoluene and about 60 mL of methylene chloride, and sonicate to dissolve. Dilute to volume with methylene chloride.

  Standard solution: Transfer 2.0 mL of the *Standard stock solution* into a 200-mL volumetric flask and dilute to volume with cyclohexane.

  Sample stock solution: Transfer 25 mg of sample into a 100-mL volumetric flask, add about 25 mg of butylated hydroxytoluene and about 60 mL of methylene chloride, and sonicate to dissolve. Dilute to volume with methylene chloride.

  Sample solution: Transfer 2.0 mL of the *Sample stock solution* into a 200-mL volumetric flask and dilute to volume with cyclohexane.

  Analysis: Using a suitable UV/VIS spectrophotometer, examine the *Sample solution* and the *Standard solution* in 1-cm cells over a spectral range of 300 to 700 nm. Record and compare the spectra from each solution.

  Acceptance criterion: The ratio of A_{476}/A_{508} is between 1.10 and 1.14.

- **B. Procedure**

  Acceptance criterion: The retention time of the major peak in the chromatogram of the *Sample solution* corresponds to that in the chromatogram of the *Standard solution* as obtained in the test for *Content of all-E-Lycopene, 5Z-Lycopene, and Related Compounds* (below).

ASSAY

- **Procedure**

  Sample stock solution: Transfer 25 mg of sample into a 100-mL volumetric flask, add about 25 mg of butylated hydroxytoluene and about 60 mL of methylene chloride, and sonicate to dissolve. Dilute to volume with methylene chloride.

  Sample solution: Transfer 2.0 mL of the *Sample stock solution* into a 200-mL volumetric flask and dilute to volume with cyclohexane.

  Analysis: Determine the absorbance of the *Sample solution* at the wavelength of maximum absorbance at about 476 nm using cyclohexane as the blank. Calculate the percent lycopene in the portion of the sample taken by the formula:

$$1000A / 31W$$

  A = Absorbance of the *Sample solution*

  W = Weight of the sample (g) taken to make the *Sample stock solution*

  331 = Absorptivity of pure lycopene in cyclohexane

  Acceptance criterion: NLT 96.0% and NMT 101.0% of lycopene ($C_{40}H_{56}$), calculated on the dried basis

IMPURITIES

Inorganic Impurities

- **Lead,** *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method II,* Appendix IIIB

  Acceptance criterion: NMT 1 mg/kg

Organic Impurities

- **Organic Volatile Impurities**

  Standard solution: 1.0 µg/mL of 1,4-dioxane, 12.0 µg/mL of ethanol, 6.0 µg/mL of methanol, and 1.0 µg/mL of *n*-propyl alcohol in dimethylformamide. Pipet 5 mL of this solution into a vial that contains 1 g of anhydrous sodium sulfate and that is fitted with a septum and a crimp cap, seal, and heat at 80° for 1 h.

  Sample solution: Transfer 100 mg of sample into a vial, add 5.0 mL of dimethylformamide and 1 g of anhydrous sodium sulfate, and seal with a septum and crimp cap. Heat the sealed vial at 80° for 1 h.

  Chromatographic system, Appendix IIA

  Mode: Gas chromatography

  Detector: Flame ionization detector

  Column: 30-m × 0.53-mm (id) column coated with 3.0-µm 6% cyanopropylphenyl-94% dimethylpolysiloxane (DB-624, J&W, or equivalent), and a 5-m × 0.53-mm (id) silica guard column deactivated with phenylmethylsiloxane

  Temperature

  Column: Hold at 40° for 20 min, then increase rapidly to 240° and hold for 20 min

Injection port: 140°
Detector: 260°
Carrier gas: Helium
Linear velocity: About 35 cm/s
Injection volume: About 1 µL
System suitability
Sample: *Standard solution*
Suitability requirement 1: All of the components in the *Standard solution* are resolved in the chromatogram.
Suitability requirement 2: The resolution, *R*, between any two components is NLT 3.
Suitability requirement 3: The relative standard deviation of the individual peak responses from replicate injections is NMT 15%.
Analysis: Separately inject the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the peak responses. Based on the retention time, identify any peaks present in the chromatogram of the *Sample solution*. Use mass spectrometry to establish the presence and identity of any peaks in the chromatogram from any of the organic volatile impurities listed below or from some other volatile impurity eluting with a comparable retention time by mass spectrometric relative abundance methods or by using a second validated column containing a different stationary phase.
Acceptance criteria
1,4-Dioxane: NMT 50 mg/kg
Ethanol: NMT 600 mg/kg
Methanol: NMT 300 mg/kg
Propyl alcohol: NMT 50 mg/kg

SPECIFIC TESTS

- **CONTENT OF ALL *E*-LYCOPENE, *5Z*-LYCOPENE, AND RELATED COMPOUNDS**
Mobile phase: *tert*-butyl methyl ether:methanol:tetrahydrofuran [784 : 665 : 74] (v/v/v), filtered and degassed. [NOTE: Adjust as necessary. See *System suitability* below.]
Standard solution: 20 µg/mL of lycopene using lycopene reference standard (Chromadex, Inc., Santa Ana, CA; Sigma, St. Louis, MO, or equivalent) in *Mobile phase*
Sample: 15 mg
Sample stock solution: Transfer the *Sample* into a 25-mL volumetric flask, dissolve in and dilute to volume with tetrahydrofuran containing 50 mg of butylated hydroxytoluene per L.
Sample solution: Pipet 2 mL of the *Sample stock solution* into a 50-mL volumetric flask, add 8 mL of tetrahydrofuran, and dilute to volume with *tert*-butyl methyl ether.
Chromatographic system, Appendix IIA
Mode: High-performance liquid chromatography
Detector: 472 nm
Column: 25-cm × 4.6-mm (id) column containing 5-µm C30, polymerically bonded, non-end-capped packing, preceding in series by a guard column containing 3-µm C30 packing
Flow rate: 1.0 mL/min
Injection volume: About 10 µL
System suitability
Sample: *Standard solution*
Suitability requirement 1: The resolution, *R*, between all-*E*-lycopene and *5Z*-lycopene is NLT 1.0.
Suitability requirement 2: The tailing factor is NLT 0.8 and NMT 2.0.
Suitability requirement 3: The relative standard deviation for replicate injections for the all-*E*-lycopene is NMT 2.0%.
[NOTE: New columns may require up to 30 injections before the system suitability requirements are met.]
Analysis: Inject the *Sample solution* into the chromatograph, record the chromatogram, and measure the peak area responses. [NOTE: Relative retention times are about 1.07 for *5Z*-lycopene and 1.0 for all-*E*-lycopene.] Calculate the percentage of related compounds in the portion of the *Sample* taken by the formula:

$$T(r_S/r_T)$$

T = Percent total lycopene isomers obtained in the *Assay* (above)
r_S = Sum of the responses of all peaks, excluding the peak for all-*E*-lycopene and the peak for *5Z*-lycopene
r_T = Total detected area
Calculate the percent *5Z*-lycopene isomer in the portion of the sample taken by the formula:

$$T(r_{5Z}/r_T)$$

T = Percent total lycopene isomers obtained in the *Assay* (above)
r_{5Z} = Peak response for *5Z*-lycopene isomer
r_T = Total detected area
Calculate the percent all-*E*-lycopene taken by the formula:

$$T(r_E/r_T)$$

T = Percent total lycopene isomers obtained in the *Assay* (above)
r_E = Peak response for all-*E*-lycopene isomer
r_T = Total detected area
Acceptance criteria
5Z-lycopene: NMT 23.0%
all-*E*-lycopene: NLT 70%
Other related compounds: NMT 9.0%
- **LOSS ON DRYING,** Appendix IIC (40° over phosphorus pentoxide, under vacuum for 4 h)
Acceptance criterion: NMT 0.2%
- **RESIDUE ON IGNITION (SULFATED ASH),** Appendix IIC
Analysis: 2 g
Acceptance criterion: NMT 0.2%

OTHER REQUIREMENTS

- **LABELING** Label as Lycopene, Synthetic.

Similac® formulas for every baby's nutritional needs

Use as directed by your baby's doctor.

Advance	Sensitive	Expert Care
Complete nutrition for the 1st year	For babies with fussiness, gas, & spit-up	For babies needing extra TLC
Advance®	Sensitive	Alimentum®
Organic	Spit-Up	NeoSure®
	Soy	Diarrhea

And **Similac Go & Grow®** for toddlers learning to eat table food

Our **Feeding Expert hotline** is available to help you with feeding questions: **800-986-8800**

SAMPLE UPC 0 70074 59884 0

Breast milk is recommended. If you choose to use infant formula, the makers of Similac have a formula that's right for your baby.

Filled by volume to 1 Quart. Extra space in bottle allows for shaking and easy pouring.

Have product-related questions?
Call 1-800-515-7677,
8:30 am–5:00 pm,
Eastern time, weekdays.
www.Similac.com

#59883 40072 8954-01

Abbott

DHA/ARA
BIRTH TO 12 MONTHS
MILK-BASED

READY TO FEED — DO NOT ADD WATER

BRAND FED IN #1 HOSPITALS

Similac®
ADVANCE®

IMMUNE SUPPORT
Early Shield®
BRAIN & EYE
STRONG BONES
LUTEIN & DHA

USDA ORGANIC

Organic
Complete Nutrition

For Your Baby's 1st Year

Infant Formula with Iron

1 QT (946 mL)

NUTRIENTS PER 100 CALORIES (5 FL OZ)

PROTEIN	2.07	G	WATER	133	G
FAT	5.40	G	LINOLEIC ACID	860	MG
CARBOHYDRATE	10.9	G			

VITAMINS

VITAMIN A	300	IU	NIACIN	1050	MCG
VITAMIN D	60	IU	FOLIC ACID (FOLACIN)	15	MCG
VITAMIN E	1.5	IU	PANTOTHENIC ACID	450	MCG
VITAMIN K	8	MCG	BIOTIN	4.4	MCG
THIAMIN (VIT. B_1)	100	MCG	VITAMIN C (ASCORBIC ACID)	9	MG
RIBOFLAVIN (VIT. B_2)	150	MCG	CHOLINE	16	MG
VITAMIN B_6	60	MCG	INOSITOL	4.7	MG
VITAMIN B_{12}	0.25	MCG			

MINERALS

CALCIUM	78	MG	COPPER	90	MCG
PHOSPHORUS	42	MG	IODINE	6	MCG
MAGNESIUM	6	MG	SELENIUM	1.8	MCG
IRON	1.8	MG	SODIUM	24	MG
ZINC	0.75	MG	POTASSIUM	106	MG
MANGANESE	5	MCG	CHLORIDE	65	MG

INGREDIENTS: WATER, **ORGANIC** NONFAT MILK, **ORGANIC** MALTODEXTRIN, **ORGANIC** SUGAR, **ORGANIC** HIGH OLEIC SUNFLOWER OIL, **ORGANIC** SOY OIL, **ORGANIC** COCONUT OIL; **LESS THAN 0.5% OF:** C. COHNII OIL*, M. ALPINA OIL†, BETA-CAROTENE, LUTEIN, LYCOPENE, FRUCTOOLIGOSACCHARIDES, POTASSIUM CITRATE, CALCIUM CARBONATE, ASCORBIC ACID, SOY LECITHIN, CARRAGEENAN, MAGNESIUM CHLORIDE, SALT, FERROUS SULFATE, CHOLINE CHLORIDE, CHOLINE BITARTRATE, TAURINE, m-INOSITOL, d-ALPHA-TOCOPHERYL ACETATE, L-CARNITINE, ZINC SULFATE, NIACINAMIDE, CALCIUM PANTOTHENATE, RIBOFLAVIN, VITAMIN A PALMITATE, CUPRIC SULFATE, THIAMINE CHLORIDE HYDROCHLORIDE, PYRIDOXINE HYDROCHLORIDE, FOLIC ACID, MANGANESE SULFATE, PHYLLOQUINONE, BIOTIN, POTASSIUM IODIDE, SODIUM SELENATE, VITAMIN D_3, CYANOCOBALAMIN, POTASSIUM HYDROXIDE AND NUCLEOTIDES (ADENOSINE 5'-MONOPHOSPHATE, CYTIDINE 5'-MONOPHOSPHATE, DISODIUM GUANOSINE 5'-MONOPHOSPHATE, DISODIUM URIDINE 5'-MONOPHOSPHATE).
CONTAINS MILK AND SOY INGREDIENTS.
*SOURCE OF DOCOSAHEXAENOIC ACID (DHA) †SOURCE OF ARACHIDONIC ACID (ARA)

USE AS DIRECTED BY A DOCTOR
Directions for Preparation and Use

USE BY DATE ON BOTTLE • DO NOT ADD WATER
DO NOT USE IF BAND AROUND CAP OR INNER FOIL SEAL IS DAMAGED.

Your baby's health depends on carefully following these directions. Failure to follow these directions could result in severe harm. Ask your baby's doctor if you need to boil (sterilize) bottles, nipples and rings before use.

Use

1. **Shake very well before each use**
   Remove protective band; twist off and clean cap
2. **Invert cap; press down to pierce foil, then turn cap a half turn**
   Remove foil
3. **Pour formula into bottle; attach nipple**
   Once feeding begins, use within 1 hour or discard

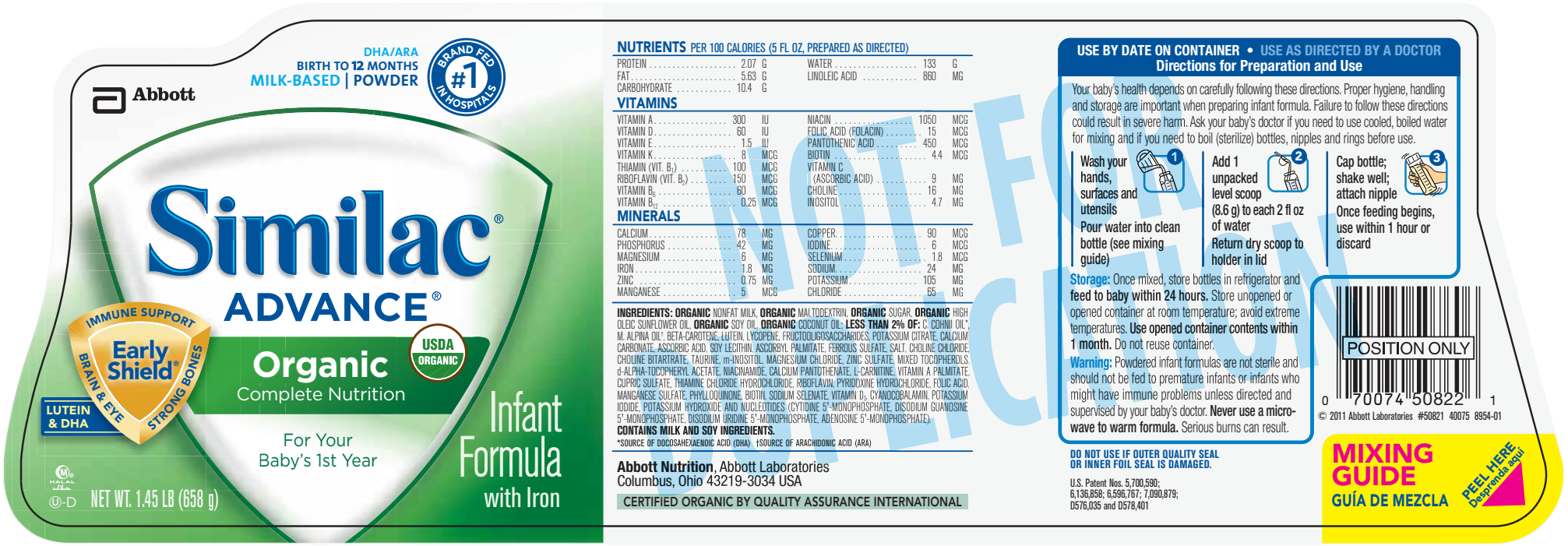
Prepare and feed all formula in this container within 3 days after opening.

Storage Once opened, store quart bottle in refrigerator. Store prepared bottles in refrigerator and **feed to baby within 48 hours.** Store unopened containers at room temperature; avoid extreme temperatures. Do not reuse container.

Warning Do not use this container to warm formula. **Never use a microwave to warm formula.** Serious burns can result.

Abbott Nutrition, Abbott Laboratories, Columbus, Ohio 43219-3034 USA

CERTIFIED ORGANIC BY QUALITY ASSURANCE INTERNATIONAL



NUTRIENTS PER 100 CALORIES (5 FL OZ, PREPARED AS DIRECTED)

PROTEIN	2.07	G	WATER	133	G
FAT	5.63	G	LINOLEIC ACID	860	MG
CARBOHYDRATE	10.4	G			

VITAMINS

VITAMIN A	300	IU	NIACIN	1050	MCG
VITAMIN D	60	IU	FOLIC ACID (FOLACIN)	15	MCG
VITAMIN E	1.5	IU	PANTOTHENIC ACID	450	MCG
VITAMIN K	8	MCG	BIOTIN	4.4	MCG
THIAMIN (VIT. B_1)	100	MCG	VITAMIN C (ASCORBIC ACID)	9	MG
RIBOFLAVIN (VIT. B_2)	150	MCG	CHOLINE	16	MG
VITAMIN B_6	60	MCG	INOSITOL	4.7	MG
VITAMIN B_{12}	0.25	MCG			

MINERALS

CALCIUM	78	MG	COPPER	90	MCG
PHOSPHORUS	42	MG	IODINE	6	MCG
MAGNESIUM	6	MG	SELENIUM	1.8	MCG
IRON	1.8	MG	SODIUM	24	MG
ZINC	0.75	MG	POTASSIUM	105	MG
MANGANESE	5	MCG	CHLORIDE	65	MG

INGREDIENTS: ORGANIC NONFAT MILK, **ORGANIC** MALTODEXTRIN, **ORGANIC** SUGAR, **ORGANIC** HIGH OLEIC SUNFLOWER OIL, **ORGANIC** SOY OIL, **ORGANIC** COCONUT OIL; **LESS THAN 2% OF:** C. COHNII OIL*, M. ALPINA OIL†, BETA-CAROTENE, LUTEIN, LYCOPENE, FRUCTOOLIGOSACCHARIDES, POTASSIUM CITRATE, CALCIUM CARBONATE, ASCORBIC ACID, SOY LECITHIN, ASCORBYL PALMITATE, FERROUS SULFATE, SALT, CHOLINE CHLORIDE, CHOLINE BITARTRATE, TAURINE, m-INOSITOL, MAGNESIUM CHLORIDE, ZINC SULFATE, MIXED TOCOPHEROLS, d-ALPHA-TOCOPHERYL ACETATE, NIACINAMIDE, CALCIUM PANTOTHENATE, L-CARNITINE, VITAMIN A PALMITATE, CUPRIC SULFATE, THIAMINE CHLORIDE HYDROCHLORIDE, RIBOFLAVIN, PYRIDOXINE HYDROCHLORIDE, FOLIC ACID, MANGANESE SULFATE, PHYLLOQUINONE, BIOTIN, SODIUM SELENATE, VITAMIN D_3, CYANOCOBALAMIN, POTASSIUM IODIDE, POTASSIUM HYDROXIDE AND NUCLEOTIDES (CYTIDINE 5'-MONOPHOSPHATE, DISODIUM GUANOSINE 5'-MONOPHOSPHATE, DISODIUM URIDINE 5'-MONOPHOSPHATE, ADENOSINE 5'-MONOPHOSPHATE).
CONTAINS MILK AND SOY INGREDIENTS.
*SOURCE OF DOCOSAHEXAENOIC ACID (DHA) †SOURCE OF ARACHIDONIC ACID (ARA)

Abbott Nutrition, Abbott Laboratories
Columbus, Ohio 43219-3034 USA

CERTIFIED ORGANIC BY QUALITY ASSURANCE INTERNATIONAL

USE BY DATE ON CONTAINER • USE AS DIRECTED BY A DOCTOR

Directions for Preparation and Use

Your baby's health depends on carefully following these directions. Proper hygiene, handling and storage are important when preparing infant formula. Failure to follow these directions could result in severe harm. Ask your baby's doctor if you need to use cooled, boiled water for mixing and if you need to boil (sterilize) bottles, nipples and rings before use.

1. Wash your hands, surfaces and utensils. Pour water into clean bottle (see mixing guide)
2. Add 1 unpacked level scoop (8.6 g) to each 2 fl oz of water. Return dry scoop to holder in lid
3. Cap bottle; shake well; attach nipple. Once feeding begins, use within 1 hour or discard

Storage: Once mixed, store bottles in refrigerator and **feed to baby within 24 hours.** Store unopened or opened container at room temperature; avoid extreme temperatures. **Use opened container contents within 1 month.** Do not reuse container.

Warning: Powdered infant formulas are not sterile and should not be fed to premature infants or infants who might have immune problems unless directed and supervised by your baby's doctor. **Never use a microwave to warm formula.** Serious burns can result.

DO NOT USE IF OUTER QUALITY SEAL OR INNER FOIL SEAL IS DAMAGED.

U.S. Patent Nos. 5,700,590; 6,136,858; 6,596,767; 7,090,879; D576,035 and D578,401

POSITION ONLY

0 70074 50822 1

MIXING GUIDE
GUÍA DE MEZCLA

PEEL HERE Despréndase aquí

BASF
The Chemical Company

Safety data sheet
LycoVit® Dispersion 10%

Revision date : 2005/09/01 Page: 1/5
Version: 1.2 (30264388/MDS_GEN_US/EN)

1. Substance/preparation and company identification

Company
BASF Corporation
100 Campus Drive
Florham Park, NJ 07932

24 Hour Emergency Response Information
CHEMTREC: (800) 424-9300
BASF HOTLINE: (800) 832-HELP

2. Composition/information on ingredients

CAS Number	Content (W/W)	Chemical name
8001-21-6	89.5 %	Sunflower oil
502-65-8	10.5 %	Lycopin krist.

3. Hazard identification

Emergency overview

CAUTION: NO PARTICULAR HAZARDS KNOWN.
Avoid contact with the skin, eyes and clothing.
Use with local exhaust ventilation.
Wear chemical resistant protective gloves.
Wear protective clothing.
Eye wash fountains and safety showers must be easily accessible.

Potential health effects

Primary routes of exposure
Routes of entry for solids and liquids include eye and skin contact, ingestion and inhalation. Routes of entry for gases include inhalation and eye contact. Skin contact may be a route of entry for liquified gases.

4. First-aid measures

General advice:
Remove contaminated clothing.

If inhaled:
Keep patient calm, remove to fresh air.

If on skin:
Wash thoroughly with soap and water.

If in eyes:
Wash affected eyes for at least 15 minutes under running water with eyelids held open.

If irritation develops, seek immediate medical attention.

BASF
The Chemical Company

Safety data sheet
LycoVit® Dispersion 10%

If swallowed:
Rinse mouth and then drink plenty of water.

Seek medical attention.

5. Fire-fighting measures

Flash point:	approx. 139 °C	(DIN 51758)
Autoignition:	370 °C	(DIN 51794)

Suitable extinguishing media:
foam, dry powder

Protective equipment for fire-fighting:
Wear a self-contained breathing apparatus.

Further information:
Dispose of fire debris and contaminated extinguishing water in accordance with official regulations.

NFPA Hazard codes:
Health : 1 Fire: 0 Reactivity: 0 Special:

6. Accidental release measures

Personal precautions:
No special precautions necessary.

Environmental precautions:
Do not discharge into drains/surface waters/groundwater.

7. Handling and storage

Handling

General advice:
Handle in accordance with good industrial hygiene and safety practice.

Protection against fire and explosion:
Risk of self-ignition when a large surface area is produced due to fine dispersion. Prevent electrostatic charge - sources of ignition should be kept well clear - fire extinguishers should be kept handy.

Storage

General advice:
Keep container tightly closed and dry; store in a cool place. Protect from the effects of light. Keep under nitrogen.

Storage stability:
Storage temperature: 0 - 25 °C

Temperature tolerance

BASF
The Chemical Company

Safety data sheet
LycoVit® Dispersion 10%

Protect from temperatures above: 50 °C
The packed product must be protected against exceeding the indicated temperature.

8. Exposure controls and personal protection

Personal protective equipment

Respiratory protection:
Wear respiratory protection if ventilation is inadequate.

Eye protection:
Tightly fitting safety goggles (chemical goggles).

Body protection:
Body protection must be chosen based on level of activity and exposure.

General safety and hygiene measures:
Handle in accordance with good industrial hygiene and safety practice.

9. Physical and chemical properties

Form:	oily	
Odour:	odourless	
Colour:	red violet	
solidification temperature:	-35 - -15 °C	
Density:	0.92 g/cm3	(20 °C)
Partitioning coefficient n-octanol/water (log Pow):	> 6	(calculated)
Solubility in water:		insoluble

10. Stability and reactivity

Hazardous reactions:
No hazardous reactions if stored and handled as prescribed/indicated.

Decomposition products:
Hazardous decomposition products: No hazardous decomposition products if stored and handled as prescribed/indicated.

Thermal decomposition:
180 °C

11. Toxicological information

Acute toxicity

Oral:
LD50/rat/male/female: > 5,000 mg/kg (OECD Guideline 423)
The product has not been tested. The statement has been derived from products of a similar structure and composition.

The Chemical Company

Safety data sheet
LycoVit® Dispersion 10%

Skin irritation:
rabbit: non-irritant (OECD Guideline 404)
The statement for skin irritation was derived from products of similar composition.

Eye irritation :
rabbit: non-irritant (OECD Guideline 405)
The product has not been tested. The statement has been derived from products of a similar structure and composition.

12. Ecological information

Environmental fate and transport

Biodegradation:

Evaluation:	Not readily biodegradable (by OECD criteria). Biodegradable. The product has not been tested. The statement has been derived from the properties of the individual components.

13. Disposal considerations

Waste disposal of substance:
Dispose of in accordance with national, state and local regulations.

14. Transport information

Reference Bill of Lading

15. Regulatory information

Federal Regulations

Registration status:

TSCA, US	released / exempt

OSHA hazard category: No data available.

SARA hazard categories (EPCRA 311/312): Not hazardous

16. Other information

HMIS III rating
Health: 1 Flammability: 0 Physical hazard: 0

The Chemical Company

Safety data sheet
LycoVit® Dispersion 10%

HMIS uses a numbering scale ranging from 0 to 4 to indicate the degree of hazard. A value of zero means that the substance possesses essentially no hazard; a rating of four indicates high hazard.

Local contact information

prod_reg@basf.com

END OF DATA SHEET

LycoVit® Dispersion 10%

BASF
The Chemical Company

Chemical name of active ingredient
Lycopene

CAS-No.	502-65-8
EINECS-No.	207-949-1

PRD-No.
30264388*

* The product is kosher.

Article

51588597	25 kg steel drum

Country of origin
Germany

Description
LycoVit Dispersion 10% is a red-violet, oily dispersion containing microcrystalline lycopene in sunflower oil. No additional stabilizers are used in the production of the dispersion.

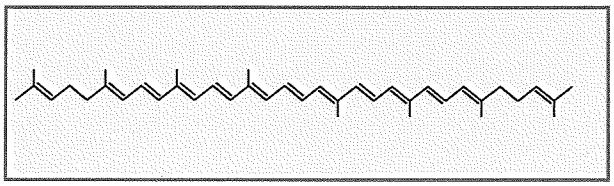
Composition
Ingredients in descending order of weight: sunflower oil, lycopene.

Specification

Assay	min. 10% lycopene

For further information see separate document: "Standard Specification" (not for regulatory purposes) available via BASF's WorldAccount: https://worldaccount.basf.com (registered access).

Monographs
The product complies with the current "Lycopene preparation" USP monograph. The active ingredient complies with the current "Lycopene" USP, FCC, and JECFA monographs.



$C_{40}H_{56}$ Molar mass 536.9 g/mol

Regulations
Lycopene is a conditionally approved food colorant and nutritional ingredient. Therefore, national regulations in the respective countries have to be observed.

Stability
Stored in its unopened original packaging at room temperature (max. 25 °C), the product is stable for at least 36 months. As microcrystalline lycopene may sink to the bottom of the packaging during storage, the dispersion should always be stirred prior to use.

Storage/Handling
The product is sensitive to atmospheric oxygen, light, heat, and moisture, and should therefore be stored under nitrogen in the tightly sealed original packaging in a cool place (8 – 15 °C). Once opened, it is recommended to use the remaining content as quickly as possible.

Applications

In dietary supplements, the lycopene dispersion is used as an active ingredient in soft gelatin capsules.

Furthermore, it is suitable for the fortification of food and supplements, especially within oily preparations.

Note

LycoVit Dispersion 10% must be handled in accordance with the Safety Data Sheet.

November 2009

CERTIFICATE

DQS GmbH
Deutsche Gesellschaft zur Zertifizierung von Managementsystemen

hereby certifies that the company

BASF SE
Operating Division Care Chemicals

67056 Ludwigshafen
Germany

has implemented and maintains an **Environmental Management System**.

Scope:
Development, manufacturing and marketing of ingredients and additives for food and feed, cosmetic ingredients, aroma chemicals, superabsorbents and performance chemicals for detergents and formulators

Through an audit, documented in a report, it was verified that the management system fulfills the requirements of the following standard:

ISO 14001 : 2004

Certificate registration no.	467055 UM
Excerpt from Certificate Registration No.	019089 UM
Date of certification	2009-12-02
Valid until	2012-12-01

TGA-ZM-02-90

Michael Drechsel
Managing Director

Jan Böge
Managing Director

August-Schanz-Straße 21, 60433 Frankfurt am Main

Annex to Certificate
Registration No. 467055 UM

BASF SE
Operating Division Care Chemicals

67056 Ludwigshafen
Germany

Location	Scope
092373 **BASF SE** **Global Business Unit** **Global Hygiene, Home & Personal Care Businesses** 67056 Ludwigshafen Germany	Development, manufacturing and marketing of superabsorbents
002854 **BASF SE** **Global Business Unit** **Nutrition Ingredients** 67056 Ludwigshafen Germany	Development, manufacturing and marketing of ingredients and additives for food and feed
467339 **BASF SE** **Global Business Unit** **Citral & Aroma Chemicals** 67056 Ludwigshafen Germany	Manufacturing and marketing of Aroma Chemicals
467054 **BASF SE** **Regional Business Unit** **Care Chemicals and Formulators Europe** 67056 Ludwigshafen Germany	Development, manufacturing and marketing of cosmetic ingredients and performance chemicals for detergents and formulators

This annex (edition: 2009-12-02) is only valid in connection with the above-mentioned certificate.

European Food Safety Authority

The EFSA Journal (2008) 676, 1-25

Safety of Synthetic Lycopene [1]

Scientific Opinion of the Panel on Scientific Panel on Dietetic Products, Nutrition and Allergies

(Question No EFSA-Q-2007-119)

Adopted on 10 April 2008 by written procedure

PANEL MEMBERS

Jean-Louis Bresson, Albert Flynn, Marina Heinonen, Karin Hulshof, Hannu Korhonen, Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Andreu Palou, Hildegard Przyrembel, Seppo Salminen, John Sean Strain, Stephan Strobel, Inge Tetens, Henk van den Berg, Hendrik van Loveren, and Hans Verhagen.

SUMMARY

Following a request from European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver a scientific opinion on the safety of synthetic lycopene for use as a novel food ingredient taking into account the various EFSA opinions on all forms and proposed uses of lycopene.

The applicant proposes to use synthetic lycopene both as a food supplement and as a food ingredient. The novel food ingredient consists of synthetic (crystalline) lycopene to be marketed in three different formulations. These are lycopene 10 %, lycopene 10 cold water dispersion (CWD) and lycopene dispersion 20 %.

Synthetic lycopene is suggested by the applicant to be used in food supplements at levels of 8 or 15 mg/dosing, in beverages and dairy products at levels of up to 2.5 mg/100 g, in breakfast cereals up to 4 mg/100 g, in cereal bars up to 8 mg/100 g, in fats and dressings up to 4 mg/ 100 g and in dietary foods for special medical purposes at levels in accordance with the particular requirements of the person for whom the products are intended.

The applicant provides an intake estimate of lycopene based on three sources including 1) normal dietary intake from food, 2) intake from dietary supplements and 3) intake from proposed fortified food products. The Panel notes that an additional source is 4) use as a food colour.

[1] For citation purposes: Scientific Opinion of the Panel on Panel on Dietetic Products, Nutrition and Allergies on a request from the European Commission on the safety of synthetic lycopene. *The EFSA Journal* (2008) 676, 1-25

An overview of average dietary intakes of lycopene from foods in different populations was presented in previous EFSA evaluations. It was concluded that regular intakes of lycopene from natural dietary sources in different populations are, according to dietary surveys, estimated to be on average between 0.5 and 5 mg/day, with high exposures up to about 8 mg/day. High consumption of fruits and vegetables, especially tomato products, may result in occasional intakes of 20 mg lycopene/day or more.

The applicant indicates that intake of lycopene from supplements is not expected to be more than 21 mg/day among supplement users, based on the combined use of one lycopene supplement (providing maximal 20 mg of lycopene) and one multi-vitamin supplement (providing max 1 mg of lycopene).

Lycopene intake via lycopene fortified products is estimated by the applicant to be 28-30 mg/day for children up to 9y, 37 mg/day for males and 33 mg/day for females aged 10-18y, and 25 mg/day for males and 23 mg/day for females over 19y. Expressed per kg bw the estimated 95th percentile intakes will be highest for children (1-3y), being 2.2 mg/kg bw/day, intermediate for children (4-9y), 1.3 and 1.4 mg/kg bw/day, for girls and boys respectively, and lowest for men and women (19y) 0.32 and 0.35 mg/kg bw/day, respectively.

In a recent opinion the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food noted that total daily exposure to lycopene as a food colour could potentially range from 2 to 6 mg on average and up to 23 mg at the high level. The AFC Panel did not exclude an occasionally combined high exposure from both natural dietary sources and food colours up to 43 mg of lycopene per day.

Based on intake estimates from all these sources the Panel concludes that intake from the proposed uses and levels of use would lead to intake levels that will substantially increase the overall dietary intake of lycopene, and could lead to daily intakes from 10.5 - 30 mg/day at the mean and to 52 - 95 mg/day as high intakes. These values amount from 0.175 - 0.5 mg/kg bw/day and 0.87 - 1.58 mg/kg bw/day for a 60 kg person and are for the high intake estimates substantially higher than the ADI recently established by the AFC Panel as a group ADI of 0.5 mg/kg bw/day for lycopene from all sources (EFSA, 2008). This ADI is in line with the ADI of 0 - 0.5 mg/kg bw/day established by JECFA (JECFA, 2006).

The Panel considers that synthetic lycopene formulated as lycopene 10% and lycopene 10 CWD is as safe as lycopene from other accepted sources.

The Panel noted that the lycopene 20 % formulation was not formulated in the presence of an antioxidant and not tested in the toxicology tests. In the absence of these data the Panel cannot conclude on the safety in use of this formulation.

The Panel concludes that for the average user consumption of synthetic lycopene and from all other sources will be below the ADI. However, some users of lycopene products may exceed the ADI of 0.5 mg/kg bw/day.

Key words:

Synthetic lycopene, novel food ingredient, CAS Registry Number 502-65-8

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE COMMISSION

On 12 October 2005, the BASF AG submitted a request under Article 4 of the Novel Food regulation (EC) No 258/97 to the competent authorities of the Netherlands for placing on the market 'synthetic lycopene' (LycoVit^{R}) as a novel food ingredient.

On 23 October 2006, the competent authorities of the Netherlands forwarded to the Commission their initial assessment report, which had reached the conclusion that 'synthetic lycopene' for the proposed uses is acceptable.

On 10 November 2006, the Commission forwarded the initial assessment report to the other Member States. Several of these Member States submitted additional comments/objections.

In consequence, a Community Decision is now required under Article 7, paragraph 1 of Regulation (EC) No 258/97.

The concerns of a scientific nature raised by the Competent Authorities of Member States can be summarised as follows:

- Objections based on uncertainties related to the insufficient information on the long-term effects of potentially high intakes of lycopene.

- It is noted that many of the foodstuffs of interest are used especially by young consumers and children, e.g. fruit juices and soft drinks, milk-based fruit beverages, sports drinks, yoghurt and breakfast cereals.

- It is noted that since several applications have been submitted on lycopene as a novel food ingredient the safety of lycopene intake should be evaluated as a whole and should include intake estimates from natural sources, potential novel food applications, additive use and use in foods for special medical purposes.

- Reproduction and teratogenicity studies provided by the applicant were based on the assumption of temporary exposure to the novel ingredients, which might not be the case in reality at least for certain people.

- It should not be added to products that may be consumed by young children (1-3 years) and one Member State would welcome discussion on labelling stating; "Not recommended for young children" from a risk management perspective.

- One may question the nutritional value of using lycopene in foodstuffs at the proposed use levels, given the phenomenon of saturation, which makes any consumption in excess of 10 mg useless.

- The safety to health of synthetic lycopene (for use as a food additive) has already been assessed by the SCF which considered the toxicological information presented at that time to be insufficient and did not accept the product for use in food stuffs (SCF, 1999). It is argued that there has been no substantial change in the toxicological data for synthetic lycopene compared with the SCFs earlier assessment.

- EFSA's Panels concluded previously that the toxicological information on lycopene was insufficient to determine an ADI and the NDA Panel in an opinion on lycopene from *Blakeslea trispora* considered that only an additional lycopene intake of 2 mg per day (but not 20 mg per day) was acceptable (EFSA, 2005a).

- In agreement with previous SCF and EFSA opinions the total lycopene intake should not substantially exceed the amount obtained from natural foods in a balanced diet. The proposed uses and use levels lead to daily intake levels at the 95th percentile in adolescents excluding food supplements, 26 times higher than the average lycopene intake obtained from non-fortified foods (52 mg as opposed to 2 mg).

- Highly concentrated lycopene is sensitive to oxygen and light and forms degradation products with mutagenic activity (SCF, 1999). Appropriate quality assurance and control measurements must be taken.

- Synthetic lycopene is often referred to as the first example of a food ingredient that will be marketed in a nanopartical form, and lycovit 10 % consists of particles < 0.5 micrometer in diameter. This aspect requires additional information to demonstrate that there are no safety concerns.

- The JECFA has set an ADI of 0 - 0.5 mg/kg bw/day. This value is 3 to 4 times lower than the proposed estimated intake for the synthetic lycopene. The toxicological data used by JECFA to set the ADI should be taken into consideration.

- The test materials used in the toxicological studies contained 2 % of related compounds of the synthetic crystalline lycopene, whereas the specifications indicate that this should be no more than 9 %. An explanation of this discrepancy between what is tested and what is in the specifications is needed.

- It is important to include studies on factors which might affect the bio-availability of lycopene, evaluating its interaction with other carotenoids present in the diet, in order to assess whether there is any difference from food naturally rich in lycopene.

- There is insufficient support for the health claims presented.

- While lycopene is also allowed as a food additive, this additional use of lycopene could lead to circumvention of the regulations made for food additives.

- The limits on use proposed by the applicant would exceed those already authorised for other sources of lycopene.

In addressing Member States' comments of a scientific nature, and considering the overall safety of synthetic lycopene the Panel has used information from the original dossier provided by the applicant, the initial assessment carried out by the authorities of the Netherlands, the comments given by the Member States, the response from the applicant to the issues raised by the Member States and the recent opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on the use of lycopene as a food additive (EFSA, 2008).

Existing authorisations and evaluations

Lycopene, extracted from tomatoes, is authorised as food colouring agent within the EU (E160d) (Directive 94/36/EC) and the US (CDR 21 73.295).

Lycopene was evaluated by the SCF in 1975 (SCF, 1975) when it was unable to allocate an ADI but felt able to accept the use of lycopene prepared from natural foods by physical processes, without further investigations, as a colouring matter in food, provided that the amount consumed did not differ significantly from the amount consumed through the relevant foodstuffs. This Opinion was reiterated in 1989 (SCF, 1989). When JECFA evaluated lycopene from natural sources in 1977 they postponed a decision because of lack of data (JECFA, 1978).

In 1999 the SCF evaluated synthetic lycopene, but the available data were not sufficient to allow for an acceptance. The SCF concluded (SCF, 1999): " The proposed specification 'not less than 96 %' lycopene is not acceptable because highly concentrated lycopene is sensitive to oxygen and light, forms degradation products with mutagenic activity, and is not identical with the beadlet formulation that has been tested toxicologically," and "The toxicological data provided on the beadlet formulation are insufficient. Therefore the Committee is not able to allocate an ADI and considers its use in food unacceptable at present."

Synthetic lycopene is currently not approved for colouring matters within the EU. It is considered generally recognised as safe (GRAS) for use as a food ingredient in the US (GRAS notice No GRN 000119).

Recently the Panel evaluated the use of an α-tocopherol-containing oil suspension of lycopene, obtained from *Blakeslea trispora* for use as a novel food (NDA, 2005). It was concluded that the α-tocopherol-containing oil suspension of lycopene obtained from *B. trispora* at an additional intake of up to about 2 mg/day is not of concern from a safety point of view. It was also concluded that this does not hold for the proposed levels of use of lycopene in foods that would give rise to an additional intake of 20 mg per day.

In July 2006, the Joint FAO/WHO Expert Evaluation Committee on Food Additives (JECFA, 2006) derived an ADI of 0 - 0.5 mg/kg bw/day based on a 104-week study in rats using a safety factor of 100 for synthetic lycopene. This ADI was made into a group ADI to include lycopene from *B. trispora*. In both cases the ADI was considered to be 0 - 0.5 mg/kg bw/day. JECFA therefore proposed that this level be considered a group ADI for lycopene. This level equates to 30 mg lycopene/day for a 60 kg individual.

The Panel noted that the ADI set by JECFA (JECFA, 2006) does not include lycopene from tomatoes. This was due to the fact that JECFA was not evaluating lycopene from tomatoes.

In a recent opinion the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (EFSA, 2008) evaluated the new toxicological data on lycopene and derived an ADI of 0.5 mg/kg bw/day using a safety factor of 100. This ADI refers to lycopene from all sources.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 9 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for 'synthetic lycopene' in the

context of Regulation (EC) No 258/97 and taking into account the various requests concerning lycopene currently under consideration.

In particular, EFSA is asked to consider the elements of a scientific nature in the comments/objections raised by the other Member States.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this opinion: Jean-Louis Bresson, Karl-Heinz Engel, Marina Heinonen, Pagona Lagiou, Bevan Moseley, Andreu Palou, Annette Pöting, Seppo Salminen, Hendrik Van Loveren, Hans Verhagen; and ad hoc expert Ivonne Rietjens.

ASSESSMENT

In accordance with the Commission Recommendation 97/618/EC, the ingredient concerned by the application belongs to Class 6. "Foods produced using a novel process". For this reason the Opinion will be an assessment of the safety data provided by the applicant to comply with the information required for novel foods of Class 6, i.e. information requirements I, II, III, IX, X, XI, XII and XIII as detailed in the following text. It does not include an assessment of the possible nutritional benefits of synthetic lycopene.

I. Specification of the novel food (NF)

Lycopene is a carotenoid with the formula $C_{40}H_{56}$. It has a molecular weight of 536.85 and the CAS Registry Number 502-65-8. Its structural formula is:

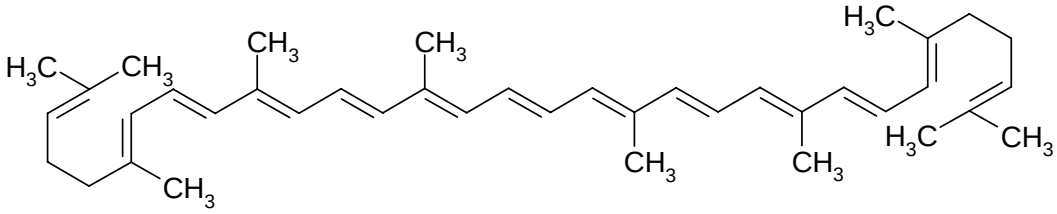


Fig 1. **Structural Formula**

Lycopene occurs in food predominantly in an all-*trans* form (Cronin, 2000; Boileau *et al.*, 2002). Tomatoes and tomato products contain the all-E (*trans*-)isomers of lycopene (between 35-96 % of total lycopene content), but also some Z (*cis*-)isomers, mainly as 5Z, 9Z, 13Z and 15Z in percentages varying between 1-22 % (Schierle *et al.*, 1997).

Synthetic lycopene consist mainly of the all *trans*-lycopene (>70 %) with 5-*cis*-lycopene (max. 20 %) and up to 3.5 % other *cis* isomers. It contains the same *cis* isomers found in tomatoes and tomato products (Table 1).

Table 1. **Cis/trans isomer ratios of lycopene in tomatoes, foodstuffs, synthetic lycopene and in human blood after consumption of lycopene (in % total lycopene).**

	Synthetic[1]	**Synthetic[2]**	**Natural[1]**	**Tomatoes[2]**	**Cooked tomato based foodstuffs[2]**	**Blood plasma[2]**
All-*trans* (%)	>70	73-77	94-96	35-96	16-96	38-40
5-*cis* (%)	Max 25	19-22	3-5	3-5	4-27	27
9-*cis* (%)	1	2-4	0-1	0-1	<1-14	4-5
13-*cis* (%)	1	1-2	1	1	1-7	7-8
Other *cis* (%)	3	-	<1	<1	<1-22	22

[1] SCF/CS/ADD/COL/160 Final 6/12/99 opinion on synthetic lycopene as a colouring matter for use in foodstuffs SCF, 1999.
[2] McClain, 2003

The novel food ingredient (LycoVit^{R}) consists of synthetic (crystalline) lycopene to be marketed as of three different formulations. These are lycopene 10 %, lycopene 10 cold water dispersion (CWD) and lycopene dispersion 20 %.

Lycopene 10 %
Lycopene 10 % is a dark red powder in which white particles of starch may be visible. The powder consists of spherical particles with a uniform particle size. The particles consist of synthetic lycopene embedded in a starch-coated matrix of fish gelatine and sucrose. They contain tocopherol (E307), sodium ascorbate (E301) and ascorbyl palmitate (E304) as antioxidants and tricalcium phosphate (E341) as an anti-caking agent. The level of synthetic lycopene is 10-12 %.

Lycopene 10 CWD
Lycopene 10 CWD is a dark red powder, which is easily dispersible in cold water. The powder consists of micronised synthetic lycopene embedded in a matrix of fish gelatine and glucose syrup. It contains DL-alpha-tocopherol (E307), ascorbyl palmitate (E304) and ascorbic acid as antioxidants. The level of the synthetic lycopene is 10-12 %.

Lycopene dispersion 20 %
Lycopene dispersion 20 % is a red-violet oily dispersion of milled synthetic crystalline lycopene. The product contains 20-22 % of the microcrystalline lycopene dispersed in food grade sunflower oil. No additional stabilizers are used in the product.

The applicant indicates that the lycopene formulations of the present opinion did not fall under the term nanoparticles. The powder particles typically have a size well above 100 micrometer. They contain lycopene particles sized about 300 nanometers on average embedded in a powder matrix. The applicant also indicates that on dissolution of the powder or in the digestive tract the powder matrix dissolves and releases the ingredient in the form of very fine emulsion droplets or suspension particles and the lycopene is subject to normal metabolism of carotenoids in the human body. The applicant also indicates that the finely dispersed carotenoid particles can be incorporated more easily into the mixed micelles containing fat, lipidoids and bile acids and that the toxicological tests performed with the synthetic lycopene formulations indicate no specific risk from the material used.

An overview of the specifications proposed by the applicant for the three lycopene formulations is given in Table 2. Synthetic crystalline lycopene is at least 96 % pure. For lycopene 10 CWD and lycopene dispersion 20 % the applicant provides the following specifications for heavy metals: heavy metals (as lead) are $\leq$ 10 mg/kg, arsenic $\leq$ 3 mg/kg and lead $\leq$ 5 mg/kg and for lycopene 10 CWD also cadmium $\leq$ 1 mg/kg and mercury $\leq$ 1 mg/kg are indicated.

Table 2. **Specifications for lycopene 10 %, lycopene 10 CWD and lycopene dispersion 20 %.**

Substance	**Composition (%)**
LycoVit® 10 %	
Synthetic crystalline lycopene	10-12
Gelatin	25-35
Sucrose	20-35
Native corn starch	20-30
Sodium ascorbate	0.5-2.5
d,l-α-tocopherol (E307)	0.5-2.0
Ascorbyl palmitate (E304)	0.1-1.2
Tri-calcium phosphate (E341)	0.4-0.6
Water	2-5

LycoVit® 10 CWD	
Synthetic crystalline lycopene	10-12
Fish gelatin	20-25
Glucose	55-60
d,l-α-tocopherol	1-2
Ascorbylpalmitate	1-2
Ascorbic acid	1-2
Tri-calcium phosphate (E341)	0.1-0.5
LycoVit® Dispersion 20 %	
Synthetic crystalline lycopene	20-22
Sunflower oil (food grade)	78-80

The applicant provided data on the stability of crystalline lycopene in the lycopene 10 % formulation at respectively 25°C and 60 % relative humidity for 36 months, of lycopene 10 CWD at 25°C and 60 % relative humidity for 12 months and at 40°C and 75 % relative humidity for 6 months, and of lycopene dispersion 20 % at 30°C and 70 % relative humidity for 9 months and at 40°C and 75 % relative humidity for 6 months. The products were stored in tightly closed containers and at each time point new aliquots containing the formulation were opened for the stability test. The results demonstrated that lycopene in these formulations and under these conditions was stable.

II. Effect of the production process applied to the NF

Synthetic lycopene is manufactured using a three stage process which is adequately described by the applicant. The ultimate product is stored and shipped in air-tight containers under inert gas. This crystalline synthetic lycopene is the starting material for the three formulations.

The other ingredients present in these formulations are common dietary ingredients or approved food additives such as cornstarch, gelatine and stabilizers such as ascorbyl palmitate and alpha-tocopherol.

Lycopene 10 %

The formulation process is carried out under a nitrogen atmosphere and using aqueous solutions containing sodium acetate and/or ascorbyl palmitate.

Lycopene 10 CWD

For applications in aqueous systems lycopene is formulated as a so-called cold water dispersible (CWD) powder in the micronization process by controlled precipitation.

Lycopene dispersion 20 %

The coarse crystalline lycopene is poured under nitrogen into sunflower oil and further processed to result in a formulation where 90 % of the crystalline lycopene particles have a diameter of less than 20 micrometer. A nitrogen stream is passed through the dispersion and the formulation is packed under nitrogen atmosphere.

The applicant indicates that the production process is not expected to result in undesirable products and that the production process and the production of the final formulations are expected to be micro-organism free.

III. History of the organism used as the source of the NF

Synthetic lycopene is obtained via chemical synthesis.

IX. Anticipated intake/extent of use of the NF

Table 3 presents the uses and use levels proposed by the applicant for lycopene in fortified foods (lycopene added to foods). Synthetic lycopene is suggested by the applicant to be used in food supplements at levels of 8 or 15 mg/dosing, in beverages and dairy products up to 2.5 mg/100 g, in breakfast cereals at up to 4 mg/100 g, in cereal bars up to 8 mg/100 g, in fats and dressings up to 4 mg/100 g and in dietary foods for special medical purposes at levels in accordance with the particular requirements of the person for whom the products are intended.

Table 3. **Uses and maximum use levels of synthetic lycopene suggested by the applicant**

Food Category	Maximum Content Level[a]
Food Supplements	
Multi-vitamin, multi-mineral tablets	8 mg/tablet
Soft gelatine capsules	15 mg/capsule
Hard gelatine capsules	15 mg/capsule
Effervescent tablets	15 mg/tablet
Waver/strips	15 mg/waver or strip
Condition-specific supplements	15 mg/tablet or capsule
Beverages	
Fruit & vegetable juice	2.5 mg/100g
Fruit juice drinks (including multi-vitamin drinks & ACE drinks)	2.5 mg/100g
Soft drinks (carbonated and non-carbonated)	2.5 mg/100g
Squash	2.5 mg/100g
Sport & Energy drinks	2.5 mg/100g
Dairy fruit drinks	2.5 mg/100g
Meal Replacements (e.g. foods intended for use in energy-restricted diets for weight reduction)	8 mg/meal replacement
Dairy Products (Except milk)	
Yoghurts, drink yoghurts	2.5 mg/100g
Breakfast Cereals	
Cereals (cold)	4 mg/100g
Cereal Bars	8 mg/100g
Fats & Dressings	
Margarine (used as spread)/ spreads	4 mg/100g

Dietary Foods for Special Medical Purposes	In accordance with the particular nutritional requirements of the persons for whom the products are intended

[a] The maximum content level of synthetic lycopene (total synthetic lycopene :cis + trans isomers) per 100 g food as prepared or tablets/capsules for supplements. Maximum content level may be provided by any of the three synthetic lycopene-containing products (LycoVit® 10 %, LycoVit® 10 CWD or LycoVit® Dispersion 20 %)

The applicant provides an intake estimate of lycopene based on three sources including 1) normal dietary intake from food, 2) intake from dietary supplements and 3) intake from proposed fortified food products. The Panel notes that an additional source is 4) use as a food colour.

Intake of lycopene from normal dietary sources

An overview of average dietary exposure to lycopene from foods in different populations was presented in previous EFSA evaluations (EFSA, 2005a and b). It was concluded that regular exposure to lycopene from natural dietary sources in different populations are, according to dietary surveys, estimated to be on average between 0.5 and 5 mg/day, with high exposures up to about 8 mg/day. High consumption of fruits and vegetables, especially tomato products, may result in occasional exposure to 20 mg lycopene/day or more.

Intake of lycopene from proposed supplement use

The applicant indicates that no data on supplement use are available, but that use of the lycopene formulations of the present opinion is likely to replace the use of other lycopene supplements. Synthetic lycopene is suggested by the applicant to be used in food supplements at levels of 8 or 15 mg/dosing.

Intake of lycopene from fortified food at the proposed uses and use levels.

For estimating the intake from fortified food products, information on the consumption of the products intended for fortification was taken from the Dutch National Food Consumption Survey that provided data on the food consumption by individuals based on a two-day dietary record method (DNFCS-3, 1998).

Tables 4 and 5 present the estimated daily intakes expressed in respectively mg/day and on a mg/kg bw/day basis provided by the applicant.

Table 4. **Estimated daily intake of lycopene (mg/day) from proposed LycoVit® fortified food products based on consumption data taken from Dutch population groups (DNFCS-3, 1998).[a]**

	All-Person intake					**All-User intake**				
Population group	N	Mean	sd	P50	P95	N	Mean	sd	P50	P95
Children, 1-3y	254	13.2	8.0	11.8	29.2	253	13.2	8.0	11.8	29.2
Boys,4-9y	242	15.9	8.3	15.0	29.6	241	16.0	8.3	15.0	29.6
Girls, 4-9y	272	15.0	7.3	14.1	28.4	272	15.0	7.3	14.1	28.4
Males, 10-18y	391	18.9	10.1	17.7	36.8	390	18.9	10.0	17.7	36.8
Females, 10-18	380	16.5	9.2	14.9	32.7	380	16.5	9.2	14.9	32.7

Males, 19+y	2117	8.8	8.5	6.5	24.9	2042	9.1	8.4	6.7	25.2
Females, 19+y	2544	8.3	8.0	6.2	22.7	2431	8.7	7.9	6.6	22.9

[a] Assuming the maximum content level is applied in the products and the consumers use only the fortified products.

Table 5. **Estimated daily intake of lycopene (mg/kg bw/day) from proposed LycoVit® fortified food products based on consumption data taken from Dutch population groups (DNFCS-3, 1998).[a]**

	All-Person intake					**All-Users intake**				
Population group	N	Mean	sd	P50	P95	N	Mean	sd	P50	P95
Children, 1-3y	254	0.98	0.61	0.89	2.2	253	0.98	0.61	0.89	2.2
Boys,4-9y	242	0.69	0.40	0.64	1.3	240	0.69	0.40	0.64	1.4
Girls, 4-9y	272	0.65	0.36	0.59	1.3	271	0.66	0.36	0.59	1.3
Males, 10-18y	391	0.35	0.20	0.33	0.68	389	0.36	0.19	0.33	0.68
Females, 10-18	380	0.33	0.19	0.31	0.67	379	0.33	0.19	0.31	0.69
Males, 19+y	2117	0.11	0.11	0.08	0.32	2041	0.11	0.11	0.09	0.32
Females, 19+y	2544	0.12	0.11	0.09	0.34	2427	0.13	0.11	0.10	0.35

[a] Assuming the maximum content level is applied in the products and the consumers use only the fortified products.

The 95th percentile values may be considered as a high intake scenario since the estimates assume that the consumers use only the fortified products. From the results presented in Tables 4 and 5 it can be concluded that the highest 95th percentile intake of added lycopene on an absolute basis is expected among males aged 10-18y, being 36.8 mg/day, equivalent to 0.68 mg/kg bw/day. On a bodyweight basis, children (1-3y) are expected to have the highest 95th percentile intake of added lycopene, being 2.2 mg/kg bw/day.

Altogether the 95th percentile lycopene intake via lycopene fortified products is estimated to be 28-30 mg/day for children up to 9y, 37 mg/day for males and 33 mg/day for females aged 10-18y, and 25 mg/day for males and 23 mg/day for females over 19y. Expressed per kg bw estimated 95th percentile intakes will be highest for children (1-3y), being 2.2 mg/kg bw/day, intermediate for children (4-9y), 1.3 and 1.4 mg/kg bw/day, for girls and boys respectively, and lowest for men and women (19y) 0.32 and 0.35 mg/kg bw/day, respectively.

Intake of lycopene resulting from use of lycopene as a food colour

An overview of average dietary exposure to lycopene from its use as a food colour in different populations was presented in a previous EFSA evaluation (EFSA, 2008). It was concluded that total daily exposure to lycopene as a food colour could potentially range from 2 to 6 mg on average and up to 23 mg at the high level.

The AFC Panel did not exclude an occasionally combined high exposure from both natural dietary sources and food colours of up to 43 mg of lycopene per day.

An overview of the estimates, compared to the exposure to naturally occurring lycopene is presented in Table 6.

Table 6. **Summary of typical lycopene exposure estimates**

Source of lycopene	Average (mg/day)	High (mg/day)	reference
Naturally occurring	0.5 - 5	8 - 20	AFC 2008
Fortified foods	8 - 19	23 – 37	Present opinion
Supplements	0 (no supplement use)	8-15	Present opinion
Food Colour	2 - 6	11 – 23*	AFC 2008

* based on the 97.5th percentile intake estimates

Overall, the Panel concludes that intake from the proposed levels of use would lead to intake levels that will substantially increase the overall dietary intake of lycopene, and could lead to mean daily intakes from 10.5 - 30 mg/day and to 52 - 95 mg/day as high intakes. The estimates for the high intakes are based on conservative assumptions. These values amount to 0.175 to 0.5 mg/kg bw/day and 0.87 - 1.58 mg/kg bw/day for a 60 kg person and are for the high intake estimates substantially higher than the ADI recently established by the AFC Panel as a group ADI of 0.5 mg/kg bw/day for lycopene from all sources (EFSA, 2008). This ADI is in line with the ADI of 0 - 0.5 mg/kg bw/day established by JECFA (JECFA, 2006).

X. Information from previous human exposure to the NF or its source

The applicant indicates that the synthetic lycopene 10 % formulation of the present opinion has been used in two small intervention studies of short duration.

In the first study 12 subjects (3 men and 9 women) used the lycopene 10 % formulation as gelatine beadlets in an amount equivalent to 15 mg total lycopene per day, for 28 days (Hoppe *et al.*, 2003). In the second study 8 postmenopausal females used synthetic lycopene in an amount equivalent to 12 mg per day for 57 days (Zhao *et al.*, 2006).

Two other human intervention studies have been published using synthetic lycopene. In one study, 6 healthy male subjects received 20 mg of lycopene (synthetic lycopene tablets) per day for 8 days (Cohn *et al.*, 2004). In the other study, 12 healthy subjects received 10 mg of synthetic lycopene per day for 12 weeks (Aust *et al.*, 2005).

No nutritional, microbial, toxicological and/or allergenicity problems were noted in these studies.

According to the applicant, supplements containing lycopene in amounts up to 20 mg are on the market in Europe and the USA.

XI. Nutritional information on the NF

The Panel considers that synthetic lycopene does not have a nutritional impact.

Bioavailability

No information on the kinetics of the synthetic lycopene formulations was provided by the applicant.

Plasma responses in man and experimental animals upon intake of synthetic lycopene or lycopene from tomatoes have been investigated frequently and data on the bioavailability of these forms of

lycopene have been presented in previous opinions from EFSA on lycopene from various sources (EFSA 2005a and b, 2008).

Lycopene absorption from purified or synthetic sources has been demonstrated to be comparable to that of tomato-based lycopene (Böhm and Bitsch 1999; Cohn *et al.*, 2004; Hoppe 2003).

In addition, interactions, both competitive and synergistic, between carotenoids have been shown to occur during the various stages of absorption (e.g., incorporation into mixed micelles, intracellular transport within enterocytes, and chylomicron assemblage), as well as during post-absorptive distribution (Furr and Clark, 1997; Van den Berg, 1999). However, the mechanisms *via* which this occurs are not clear, and definite relationships between specific carotenoids have not been established. However, as long as intake levels are within normal dietary intake, such interactions are unlikely to have a significant impact on the systemic bio-availability of an individual component.

Metabolism

Very little is known about the metabolism or degradation of lycopene in mammals (Clinton, 1998; Parker 1996). It has been shown that lycopene does not exhibit provitamin A activity (Van Vliet *et al.*, 1996; Agarwal and Rao, 2000). Furthermore, few metabolites of lycopene have been documented in human plasma or tissues. For example, two oxidative lycopene metabolites, identified as epimeric 2,6-cyclolycopene-1,5-diols, have been detected in breast milk and serum of three lactating mothers (Khachik *et al.*, 1997). It is postulated by the authors that these compounds may result via an *in vivo* metabolic oxidation of lycopene to lycopene epoxide. Upon oral administration of ^{14}C labelled lycopene to rats and monkeys no evidence for any metabolic products of lycopene was observed (McClain and Bausch, 2003).

XII. Microbiological information on the NF

Synthetic lycopene is obtained from a chemical synthetic process. The applicant indicates that the manufacturing of the formulations is a hygienic procedure performed according to HACCP, and that therefore contamination with micro-organisms is not expected.

XIII. Toxicological information on the NF

Lycopene, either natural or synthetic has been thoroughly studied in both animals and humans. Toxicological information on lycopene from different sources including lycopene from tomatoes, synthetic lycopene and lycopene from *B. trispora* has been evaluated in several previous opinions by SCF (1975, 1989, 1999), EFSA (2005; 2008) and JECFA (2006).

In July 2006, the Joint Evaluation Committee on Food Additives (JECFA, 2006) derived an ADI for synthetic lycopene and lycopene from *B. trispora* (JECFA, 2006). In both cases the ADI was considered to be 0 - 0.5 mg/kg bw/day. JECFA therefore proposed that this level be considered a group ADI for lycopene. This level equates to 30 mg lycopene/day maximum for a 60 kg individual.

Recently EFSA also evaluated the new toxicological data for lycopene and derived an ADI of 0.5 mg/kg bw/day. This ADI refers to lycopene from all sources (EFSA, 2008).

The present opinion only presents the studies specific for the synthetic lycopene formulations of the present opinion.

Acute oral toxicity

The applicant described results from an acute oral toxicity study, an acute dermal irritation/corrosion study and an acute eye irritation/corrosion study, performed with the synthetic lycopene 10 % formulation. In the acute oral toxicity study, no mortality, clinical signs, changes in body weight or abnormalities were observed. The LD50 for the test substance test substance LycoVit® 10 CWD (purity of 9.81 %), was ≥ 5000 mg/kg bw (about 500 mg lycopene/kg bw). The skin and eye irritation tests showed no toxic effects. Thus, the acute oral toxicity of the synthetic lycopene 10 % formulation is low.

Subacute and subchronic toxicity

The applicant reports a repeated dose 90-day oral toxicity study with the synthetic lycopene in rats performed according to OECD guidelines (application dossier; Mellert *et al.*, 2002). In this study, groups of 10 male and 10 female Wistar rats were exposed to 0, 500, 1500, 3000 mg/kg bw/day LycoVit® 10 CWD (purity 9.81 % lycopene) or to LycoVit® 10 % (purity 10.8 % lycopene) by gavage for 3 months.

For an interim sacrifice, additional groups of 5 male and 5 female Wistar rats were administered by gavage for 4 weeks lycopene 10 CWD at doses of 0 and 3000 mg/kg bw/day, and lycopene 10 % at 3000 mg/kg bw/day. Food consumption and body weights were determined weekly. The animals were examined for clinical signs of toxicity or mortality at least once a day. Additionally, general clinical observations were carried out before and after daily treatment. Detailed clinical examinations were conducted prior to the start of the administration and weekly thereafter. A functional observational battery (FOB) and measurement of motor activity was carried out towards the end of the 3-month administration period. Ophthalmological examinations were carried out with the animals of the 3-months administration prior to the start and towards the end of the 3 months administration period. Clinical chemistry and haematological examinations were carried out towards the end of the 4-week and 3-month administration period. Urine analysis was carried out towards the end of the 3 months administration period. All animals were assessed forgross pathology, followed by histopathological examinations.

The mean absolute thymus weight was slightly although significantly decreased after 4 weeks of application in females that received 3000 mg/kg lycopene 10 %. The applicant indicates that this was not regarded as treatment related, because a comparable effect was not noted in males and because no microscopy finding was obtained that may correlate with this weight change. Moreover, a comparable effect was not seen after the 3 month application period in either sex. The same was true for the slight but significant increased mean kidney weights after 4 weeks of application seen in males receiving 3000 mg/kg bw/day lycopene 10 CWD or 3000 mg/kg bw/day lycopene 10 %, as the females were not affected, morphology failed to detect a meaningful morphologic correlate for the increased weights and no such observation was made after the 3-month application period in either sex.

All significant weight changes, all gross lesions and all microscopic findings recorded after 4-week and 3-month application periods were either single observations or they were recorded at low or comparable incidence and graded severity in males and/or females of control and investigated treatment groups.

It was concluded that no treatment related effects were observed, except for a red discoloration of the faeces as well as the contents of the gastrointestinal tract of the animals treated with both formulations. This effect is not considered to be an adverse effect but is due to the red colour of the test substance. The no observed adverse effect level (NOAEL) was at least 3000 mg/kg bw/day, the highest dose tested for both formulations.

The applicant concludes that the NOAEL of this study amount to at least 3000 mg 10 % lycopene formulation/kg bw/day, the highest dose tested for both formulations, amounting to approximately 300 mg lycopene/kg bw/day.

An overview of other subchronic studies with oral administration of synthetic or natural lycopene or lycopene from *B. trispora* to experimental animals can be found in previous evaluations on the safety of lycopene from *B. trispora* or lycopene from tomatoes or synthetic lycopene, made by the SCF (1999), EFSA (2005, 2008) and JECFA (2006).

Reproductive toxicity and teratogenicity

No reproductive toxicity study was performed with the synthetic lycopene formulations. In the 90 day toxicity study with the synthetic lycopene 10 % and 10 CWD formulations in rats no histopathological changes were observed in the reproductive organs.

Two prenatal developmental toxicity studies were performed with the synthetic lycopene 10 % and 10 CWD formulations (Richards 2001a, Richards 2001b; Christian *et al*., 2003).

In the first study groups of 25 time-mated female KBL New Zealand White strain rabbits were exposed to synthetic lycopene 10 CWD or lycopene 10 %, vehicle or matrix control by oral gavage once a day from day 6 to day 28 post-coitum. Due to the difficulty of administering, the number of animals in some groups was increased in order to have at least 16 litters/group for foetal examination. The test substances were administered in the vehicle (purified water) at dose levels of 0 (vehicle control), 500, 1500 and 2000 mg lycopene 10 CWD/kg bw/day (amounting to 0, 50, 150 and 200 mg synthetic lycopene/day), to 2000 mg lycopene 10 %/kg bw/day (amounting to 200 mg synthetic lycopene/kg bw/day) and to placebo controls consisting of the matrix of lycopene 10 CWD at 2000 mg/kg bw, or the matrix of lycopene 10 % at 2000 mg/kg bw/day.

Clinical signs and mortality were checked daily. Body weight and food consumption were recorded at designated intervals. On day 29 post-coitum, the dams were sacrificed. A macroscopic post-mortem, which included examination of the principal thoracic and abdominal organs and the placenta, was performed. The foetuses were removed by hysterectomy. For each of the females the litter parameters, including the number of corpora lutea, implantation sites, early and late resorptions, dead and live foetuses were recorded. All the foetuses were weighed and subjected to an external examination. Fresh dissection of all live foetuses allowed a detailed examination of soft tissue. Half of the foetuses were processed for evaluation of designated tissues. The brains of the remaining half of the foetuses were examined by serial

sectioning. The carcasses of all the foetuses were fixed and detailed skeletal examination of the bone and cartilage structures was performed.

None of the test groups showed treatment related effects indicative of maternal or foetal toxicity, or influences on gestation parameters. It is concluded that the NOAEL of the study amounts to 2000 mg /kg bw/day (amounting to 200 mg synthetic lycopene/kg bw/day), the highest dose tested.

In the second study lycopene 10 CWD and lycopene 10 % were administered by daily oral gavage to pregnant female Sprague Dawley rats from implantation to 1 day prior to the expected day of parturition (day 6 to day 19 post-coitum inclusive). Groups of 25 mated female rats were exposed to 0 (vehicle control), 500, 1500 or 3000 mg lycopene 10 CWD/kg bw/day or to 3000 mg lycopene 10 %/kg bw/day or to placebo controls consisting of the matrix of lycopene 10 CWD at 3000 mg/kg bw, or the matrix of lycopene 10 % at 3000 mg/kg bw/day.

Clinical signs and mortality were checked daily. Body weight and food consumption were recorded at designed intervals. On day 20 post-coitum, the dams were sacrificed and subjected to a macroscopic examination. The pregnant uterus weight was recorded to allow the calculation of the net body weight of the dams. The foetuses were removed by hysterectomy. The litter parameters were recorded including the number of corpora lutea, implantation sites, uterine scars, early and late resorptions, dead and live foetuses. The foetuses were weighed, their sex determined and submitted to an external examination. Half of the foetuses were subjected to a detailed examination of the soft tissues and the other half of the foetuses was subjected to a detailed examination of the skeleton and cartilage.

None of the test groups showed treatment related effects indicative of maternal or foetal toxicity, or influences on gestation parameters. It is concluded that the NOAEL for the study amounts to 3000 mg /kg bw/day (amounting to 300 mg synthetic lycopene/kg bw/day), the highest dose tested.

Additional developmental and reproduction studies with oral administration of synthetic lycopene can be found in the opinion of the AFC, which describes studies revealing (EFSA, 2008):

- A NOAEL of 500 mg lycopene/kg bw/day (the highest dose level tested) in a 14-week rat study
- A NOAEL of 500 mg lycopene/kg bw/day (the highest dose level tested) in a developmental toxicity study in the rat
- A NOAEL of 500 mg lycopene/kg bw/day (the highest dose level tested) in a two-generation study in the rat
- A NOAEL of 400 mg lycopene/kg bw/day (the highest dose level tested) in a developmental toxicity study in the rabbit.

Chronic toxicity and carcinogenicity

In a most recent AFC Opinion two long term studies in rats with synthetic lycopene are described (51 and 104 weeks respectively) (EFSA, 2008).

Additional long term studies with oral administration of synthetic lycopene can be found in the AFC opinion which describes studies revealing:

- A NOAEL of 50 mg lycopene/kg bw/day in a one year rat study, and
- A NOAEL of 50 mg lycopene/kg bw/day (the highest dose level tested) in a two year rat carcinogenicity study.

Mutagenicity

The applicant provided results from genotoxicity studies performed with the lycopene 10 CWD formulation. The preparation was negative in bacterial tests with *Salmonella enterica var.* Typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 and *E.coli* WP2uvrA all with and without metabolic activation (Engelhardt and Hoffman 2000).

Synthetic lycopene 10 CWD formulation tested positive in the chromosome aberration test in Chinese hamster cells (V79 cell line) without metabolic activation, but was negative in the same test in the presence of metabolic activation (Engelhardt and Hoffman 2001b). The applicant indicates that this positive effect may be the result of an indirect mechanism due to extreme culture conditions.

No genotoxicity was observed as well for the lycopene 10 CWD formulation in two *in vivo* tests including a test for DNA repair in rat hepatocytes (Engelhardt and Hoffmann 2001c) and a mouse bone marrow micronucleus test (Engelhardt and Hoffmann 2001a).

Previously the SCF (SCF, 1999), EFSA (EFSA, 2005a; EFSA 2008) and JECFA (JECFA, 2006) have evaluated the genotoxicity of lycopene from various sources including natural lycopene from tomatoes, synthetic lycopene and lycopene from *B. trispora*, and concluded that genotoxicity data do not give reason for concern.

An important issue in the potential genotoxicity of lycopene is the degradation of pure crystalline lycopene into mutagenic products when exposed to air and light. These studies revealed that lycopene in a beadlet formulation and pure crystalline lycopene surrounded by the antioxidant alpha-tocopherol were not mutagenic (McClain and Bausch, 2003). The applicant indicates that in order to avoid degradation of lycopene in the synthetic lycopene formulations, several measures are taken, such as the addition/presence of antioxidants and specific storage conditions.

Altogether it is concluded that the present database on genotoxicity of lycopene from various sources indicates that there is no reason for concern with respect to genotoxicity.

Allergenicity and Irritancy

The skin and eye irritation tests with the synthetic lycopene 10 % formulation showed no toxic effects.

According to the applicant, there is no information in public literature on allergenic reactions associated with lycopene and no information was submitted on the possible allergenic potential of (synthetic) lycopene. In theory an allergic reaction due to the additive fish gelatine in the synthetic lycopene 10 CWD formulation is possible. However, the applicant indicates that in a double-blind placebo-controlled food challenge study of 30 patients with clinical allergy to fish, no patients reacted to a cumulative dose of fish gelatine, except for one patient showing a reaction at a very high dose in the gram range (EFSA, 2004).

DISCUSSION

The applicant proposes to use synthetic lycopene both as a food supplement and as a food ingredient. Synthetic lycopene is suggested by the applicant to be used in food supplements at levels of 8 or 15 mg/dosing/day, in beverages and dairy products up to 2.5 mg/100 g, in breakfast cereals up to 4 mg/100 g, in cereal bars up to 8 mg/100 g, in fats and dressings at levels up to 4 mg/100 g and in dietary foods for special medical purposes at levels in accordance with the particular requirements of the person for whom the products are intended.

The applicant provides an intake estimate of lycopene based on three sources including 1) normal dietary intake from food, 2) intake from dietary supplements and 3) intake from proposed fortified food products. The Panel notes that an additional source is 4) use as a food colour.

An overview of average dietary exposure to lycopene from foods in different populations was presented in previous EFSA evaluations (EFSA, 2005a and b). It was concluded that regular exposure to lycopene from natural dietary sources in different populations is, according to dietary surveys, estimated to be on average between 0.5 and 5 mg/day, with high exposures up to about 8 mg/day. High consumption of fruits and vegetables, especially tomato products, may result in occasional exposure to 20 mg lycopene/day or more.

The 95th percentile intake via lycopene fortified products is estimated to be 28-30 mg/day for children up to 9y, 37 mg/day for males and 33 mg/day for females aged 10 -18y, and 25 mg/day for males and 23 mg/day for females over 19y. Expressed per kg bw estimated 95th percentile intakes will be highest for children (1-3y), being 2.2 mg/kg bw/day, intermediate for children (4-9y), being 1.3 and 1.4 mg/kg bw/day, for girls and boys respectively, and lowest for men and women (19y) being 0.32 and 0.35 mg/kg bw/day, respectively.

In a recent opinion the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food noted that total daily exposure to lycopene as a food colour could potentially range from 2 to 6 mg on the average and up to 23 mg at the high level (EFSA, 2008). The AFC Panel did not exclude an occasionally combined high exposure from both natural dietary sources and food colours up to 43 mg of lycopene per day.

No mutagenic potential of formulated synthetic lycopene was observed in a battery of mutagenicity assays. Ames test data for crystalline, synthetic lycopene showed no mutagenic activity.

Synthetic lycopene 10 CWD formulation tested positive in the chromosome aberration test in Chinese hamster cells (V79 cell line) without metabolic activation, but was negative in the same test in the presence of metabolic activation. However no genotoxicity was observed for the lycopene 10 CWD formulation in two *in vivo* tests including a test for DNA repair in rat hepatocytes and a mouse bone marrow micronucleus test.

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food recently evaluated the safety of lycopene from all sources for uses and use levels as a food colour, including lycopene from tomatoes, *B. trispora* and synthetic lycopene (EFSA, 2008).

The AFC Panel concluded that the safety of synthetic lycopene was demonstrated in subchronic and chronic toxicity studies in rats, a carcinogenicity study and a two-generation study in rats, and developmental toxicity studies in the rat and rabbit. Mutagenicity has been

studied in an extensive program using formulated forms of lycopene and demonstrated no concern. The following no observed adverse effect levels (NOAELs) for formulated, synthetic lycopene, were established in guideline-conforming toxicity studies:

- 500 mg lycopene/kg bw/day (the highest dose level tested) in a 14-week rat study and in a developmental toxicity study in the rat
- 500 mg lycopene/kg bw/day (the highest dose level tested) in a two-generation study in the rat
- 400 mg lycopene/kg bw/day (the highest dose level tested) in a developmental toxicity study in the rabbit
- 50 mg lycopene/kg bw/day in a one year rat study and
- 50 mg lycopene/kg bw/day (the highest dose level tested) in a two year rat carcinogenicity study.

The NOAEL from a 90 day oral toxicity study with lycopene extracted from *B. trispora* amounted to about 600 mg/kg bw/day.

From the lowest NOAEL of 50 mg/kg bw/day the AFC Panel derived an ADI of 0.5 mg/kg bw/day using a safety factor of 100. This ADI refers to lycopene from all sources (EFSA, 2008).

This ADI is in line with the ADI of 0 - 0.5 mg/kg bw/day established by JECFA (JECFA, 2006) The Panel noted that the ADI set by JECFA does not include lycopene from tomatoes. This was probably due to the fact that JECFA was not evaluating lycopene from tomatoes.

The present opinion presents data from toxicity studies with synthetic lycopene 10 % and 10 CWD formulations. The NOAEL derived from a 90-day oral toxicity study in rats amounted to at least 3000 mg 10 % lycopene formulation/kg bw/day, the highest dose tested. The NOAELs for developmental toxicity studies in rabbits and rats amounted to respectively at least 2000 and 3000 mg 10 % lycopene formulation)/kg bw/day, the highest doses tested in both studies, amounting to approximately 300 mg lycopene/kg bw/day. The Panel notices that the NOAELs derived for these studies with the synthetic lycopene 10 % formulations are in the same range as those reported for the studies summarised in the AFC opinion (EFSA, 2008). Therefore, the Panel concludes that the data provided do not give reason for re-evaluation of the ADI recently derived.

Overall, the Panel concludes that intake from the proposed levels of use would lead to intake levels that will substantially increase the overall dietary intake of lycopene, and could lead to mean daily intakes from 10.5 - 30 mg/day at the mean and to 52 - 95 mg/day as high intakes. These values amount to 0.175 - 0.5 mg/kg bw/day and 0.87 - 1.58 mg/kg bw/day for a 60 kg person and are, for the high intake estimates, substantially higher than the ADI recently established by the AFC Panel as a group ADI of 0.5 mg/kg bw/day for lycopene from all sources (EFSA, 2008). This ADI is in line with the ADI of 0 - 0.5 mg/kg bw/day established by JECFA (JECFA, 2006).

CONCLUSIONS AND RECOMMENDATIONS

The Panel considers that synthetic lycopene formulated as lycopene 10 % and lycopene 10 CWD is as safe as lycopene from other accepted sources provided appropriate safety measures are taken to prevent oxidative deterioration of crystalline lycopene.

The Panel noted that the lycopene 20 % formulation was not formulated in the presence of an antioxidant and not tested in the toxicology tests. In the absence of these data the Panel cannot conclude on the safety in use of this formulation.

The Panel concludes that for the average user consumption of synthetic lycopene and from all other sources will be below the ADI. However, some users of lycopene products may exceed the ADI of 0.5 mg/kg bw/day.

DOCUMENTATION PROVIDED TO EFSA

1) Letter from the European Commission to the Chairman of the European Food Safety Authority with the request for an opinion on the safety of 'synthetic lycopene'. SANCO E4/Ak/bs (2007) D/540328

2) Initial assessment report by the Bureau Nieuwe Voedingsmiddelen (NL) concerning the assessment of 'synthetic lycopene',

3) Letters from Member States with comments on the initial assessment report on synthetic lycopene from Bureau Nieuwe Voedingsmiddelen (NL)

4) Response to Member States comments on the Netherland Opinion for synthetic lycopene as a novel food ingredient.

5) Application under regulation No 258-97 for the use of synthetic lycopene as a novel food ingredient.

REFERENCES

Agarwal S and Rao AV (2000). Tomato lycopene and its role in human health and chronic diseases. CMAJ 163: 739-744.

Aust O, Stahl W, Sies H, Tronnier H, Heinrich U (2005). Supplementation with Tomato-Based Products Inreases Lycopene, Phytofluene, and Phytoene Levels in Human Serum and Protects Against UV-light-induced Erythema. Int J Vitam Nutr Res 75(1): 54-60.

Boileau TW, Boileau AC, Erdman JW (2002). Bioavailability of all-*trans* and *cis*-isomers of lycopene. Exp Biol Med 227: 914-919.

Böhm V and Bitsch R (1999). Intestinal absorption of lycopene from different matrices and interactions to other carotenoids, the lipid status, and the antioxidant capacity of human plasma. Eur J Nutr 38: 118-125.

Christian MS, Schulte S, Hellwig J (2003). Developmental (embryo-fetal toxicity/teratogenicity) toxicity studies of synthetic crystalline lycopene in rats and rabbits. Food and Chemical Toxigology 41: 773-783.

Clinton SK (1998). Lycopene: chemistry, biology, and implications for human health and disease. Nutr Rev 56: 35-51.

Cohn W, Thürmann P, Tenter U, Aebischer C, Schierle J, and Schalch W (2004). Comparative multiple dose plasma kinetics of lycopene administered in tomato juice, tomato soup or lycopene tablets. Eur J Nutr 43(5): 304-312.

Cronin JR (2000). Lycopene; The powerful antioxidant that makes tomatoes red. Altern Complement Ther 6: 92-94.

DNFCS-3 (Dutch National Food Consumption Survey). http://www.rivm.nl/vcp_en/studies/ Wat eet Nederland: Resultaten van de Voedselconsumptiepeiling 1997/1998; 1998.

EFSA (2004). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to a notification from DSM on fish gelatine for use as a formulation aid (carrier) in vitamin and carotenoid preparations pursuant to Article 6 paragraph 11 of Directive 2000/13/EC. The EFSA Journal 150: 1-10.

EFSA (2005a). Opinion of the Scientific Panel on Dietetic products, Nutrition and Allergies on a request from the Commission related to an application n the use of α-tocopherol-containing oil suspension of lycopene from *Blakeslea trispora* as a novel food ingredient. The EFSA Journal 212: 1-29.

EFSA (2005b). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to an application on the use of α-tocopherol containing oil suspensions and cold water dispersible forms of lycopene from *Blakeslea trispora* as a food colour. The EFSA Journal 257: 1-17.

EFSA (2008). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, on a request from the European Commission on the use of lycopene as a food additive. The EFSA Journal 674, 1-66.

European Commission (1995). Commission Directive 95/45/EC of 26 July 1995 laying down specific purity criteria concerning colours for use in foodstuffs. Official Journal of the European Communities, L 226, 22.9.1995, p.1-41

European Commission (1997). Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. Official Journal of the European Communities L 253, 16.09.1997, p. 1-36.

European Parliament and Council (1997). Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Official Journal of the European Communities L 43, 14.2.1997, p. 1–6.

European Parliament and Council (2002). Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities L 31, 1.2.2002, p. 1–24

Furr HC and Clark RM (1997). Intestinal absoprtion and tissue distribution of catrotenoids. J Nutr Biochem 8: 364-377.

Hoppe PP (2003). Synthetic and tomato-based lycopene have identical bioavailability in humans. Eur J Nutr 42: 272-278.

JECFA (2006). Sixty-seventh meeting, Rome, 20-29 June 2006, Summary and conclusions, published July 7, 2006.

Khachik F, Spangler CJ, Smith JC, Jr., Canfield LM, Steck A, Pfander H (1997). Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. Anal Chem 69: 1873-81.

McClain RM and Bausch J (2003). Summary of safety studies conducted with synthetic lycopene. Regul Toxicol Pharmacol 37: 274-285.

Mellert W, Deckardt K, Gembardt C, Schulte S, Van Ravenzwaay B, Slesinski RS (2002). Thirteen-week oral toxicity study of synthetic lycopene products in rats. Food Chem Toxicol 40: 1581.

Parker RS (1996). Absorption, metabolism, and transport of carotenoids. FASEB J 10: 542-51.

Richard J (2001a). Report: Lycopene 10 CWD and LycoVit 10 %, Prenatal developmental toxicity study in sprague-dawley rats oral adminstration (Cavage). BASF Project No: 30R0116/009011, Volume I of III, Report section, figures and summary tables. Evreux, France.

Richard J (2001b). Report: Lycopene 10 CWD and LycoVit 10 %, Prenatal developmental toxicity study in New Zealand white rabbits oral adminstration (Cavage). BASF Project No: 40R0116/009017, Volume I of III, Report section, figures and summary tables. Evreux, France.

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

SCF (The Scientific Committee for Food) (1975). Reports of the Scientific Committee for Food (First series) Opinion expressed on 27 June 1975. Commission of the European Communities, p17-31. http://europa.eu.int/comm/food/fs/sc/scf/reports/scf_reports_01.pdf

SCF (The Scientific Committee for Food) (1989). Reports of the Scientific Committee for Food (Twenty-first series) Opinion expressed on 10 December 1987. Commission of the European Communities, p1-18. http://europa.eu.int/comm/food/fs/sc/scf/reports/scf_reports_21.pdf

SCF (The Scientific Committee for Food) (1999). Opinion on synthetic lycopene as a colouring matter for use in foodstuffs. Opinion expressed on 2 December 1999. http://ec.europa.eu/food/fs/sc/scf/out47_en.pdf

Van den Berg (1999). Carotenoid interactions. Nutr Rev 57: 1-10

Van Vliet T, van Schaik F, Schreurs WH, van den Berg H (1996). *In vitro* measurement of beta-carotene cleavage activity: methodological considerations and the effect of other carotenoids on beta-carotene cleavage. Int J Vitam Nutr Res 66: 77-85.

Zhao X, Aldini G, Johnson EJ, Rasmunssen H, Kraemer K, Woolf H, Musaeus N, Krinsky NI, Russeli RM, Yeum, K-J (2006). Modification of Lymphocyte DNA Damage by Carotenoid Supplementation in Post-menopausal Women. Am J Clin Nutr 83(1): 163-169.

DOI 10.1002/mnfr.200800053 Mol. Nutr. Food Res. 2009, *53*, S194 – S218

Review

Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans

Giuseppe Maiani[1], María Jesús Periago Castón[2], Giovina Catasta[1], Elisabetta Toti[1], Isabel Goñi Cambrodón[3], Anette Bysted[4], Fernando Granado-Lorencio[5], Begoña Olmedilla-Alonso[6], Pia Knuthsen[4], Massimo Valoti[7], Volker Böhm[8], Esther Mayer-Miebach[9], Diana Behsnilian[9] and Ulrich Schlemmer[10]

[1] Unit of Human Nutrition, National Institute for Food and Nutrition Research, Rome, Italy
[2] Department of Food Technology, Food Science and Nutrition, University of Murcia, Murcia, Spain
[3] Department of Nutrition, University Complutense of Madrid, Madrid, Spain
[4] Department of Food Chemistry, National Food Institute, Technical University of Denmark, Søborg, Denmark
[5] Unidad de Vitaminas, Servicio de Bioquímica Clínica, Hospital Universitario Puerta de Hierro, Madrid, Spain
[6] Departamento de Metabolismo y Nutrición, Instituto del Frío, Consejo Superior de Investigaciones Científicas, Madrid, Spain
[7] Institute of Pharmacological Sciences, School of Pharmacy, University of Siena, Siena, Italy
[8] Institute of Nutrition, Friedrich Schiller University Jena, Jena, Germany
[9] Department of Food and Bio Process Engineering, Max Rubner-Institute, Federal Research Institute of Nutrition and Food, Karlsruhe, Germany
[10] Federal Research Centre for Nutrition, Institute of Nutritional Physiology, Karlsruhe, Germany

Carotenoids are one of the major food micronutrients in human diets and the overall objective of this review is to re-examine the role of carotenoids in human nutrition. We have emphasized the attention on the following carotenoids present in food and human tissues: β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin; we have reported the major food sources and dietary intake of these compounds. We have tried to summarize positive and negative effects of food processing, storage, cooking on carotenoid content and carotenoid bioavailability. In particular, we have evidenced the possibility to improve carotenoids bioavailability in accordance with changes and variations of technology procedures..

Keywords: Bioavailability / Carotenoids / Epidemiological studies / Food source / Technology process

Received: February 5, 2008; revised: May 27, 2008; accepted: May 29, 2008

1 Introduction

Carotenoids are a widespread group of naturally occurring fat-soluble pigments. They are especially abundant in yellow-orange fruits and vegetables and in dark green, leafy vegetables. In plant cells, carotenoids are mainly present in lipid membranes or stored in plasma vacuols [1, 2].

Literature reports on the various aspects of the biosynthesis of carotenoids and the changes in their accumulation in plants through genetic and environmental factors. Food carotenoids have been compiled in several tables and databases, generally including provitamin A carotenoids such as β-carotene and β-cryptoxanthin, as well as others without that provitamin activity, such as lycopene and lutein, and others less studied in relation to human health such as phytoene or phytofluene [1–4].

In human beings, carotenoids can serve several important biological activities. The most widely studied and well-understood nutritional role for carotenoids is their provitamin A activity. Deficiency of vitamin A is a major cause of premature death in developing nations, particularly among children. Vitamin A, which has many vital systemic functions in humans, can be produced within the body from certain carotenoids, notably β-carotene [5].

Correspondence: Dr. Giuseppe Maiani, Via Ardeatina 546, 00178 Rome, Italy
E-mail: maiani@inran.it
Fax: +39-06-51494550

Abbreviations: FBS, food balance sheets; **FCTs,** food composition tables; **FFQ,** Food Frequency Questionnaire; **MP,** minimally processed

Carotenoids also potentially play an important role in human health by acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen. Lycopene, the hydrocarbon carotenoid that gives tomatoes their red colour, is particularly effective at quenching the destructive potential of singlet oxygen [6]. Lutein and zeaxanthin and xanthophylls found in corn and in leafy greens such as kale and spinach, are believed to function as protective antioxidants in the macular region of the human retina, protection against cataract formation, coronary heart diseases and stroke [7–9]. Astaxanthin, a xanthophyll found in salmon, shrimp and other seafoods, is another naturally occurring xanthophyll with potent antioxidant properties [10]. Other health benefits of carotenoids that may be related to their antioxidative potential, include enhancement of immune system function [11], protection from sunburn [12] and inhibition of the development of certain types of cancers [13].

In this overview, food sources and intake, effects of food processing and bioavailability have been considered.

2 Food sources and intake

2.1 Carotenoid content of foods

In developed countries, 80–90% of the carotenoid intake comes from fruit and vegetable consumption. Of the more than 700 naturally occurring carotenoids identified thus far, as many as 50 are present in the human diet and can be absorbed and metabolized by the human body [14]; however only six (β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin), representing more than 95% of total blood carotenoids, are present in the blood of people from different countries and have been studied and associated with some health benefits.

The most studied carotenoids are the following six: β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin, which are all important in human nutrition due to their biological activities. 'The Carotenoid Content of US Foods' is a comprehensive database, representative of US food consumption and including raw, processed and cooked forms, as described by Holden *et al*. in 1999 [15]. Similarly, O'Neill *et al*. [3] reported a European database covering the most commonly consumed carotenoid-rich foods in five European countries: UK, Ireland, Spain, France and The Netherlands. This database is a compilation of investigations from the 1990s. In 1995, Hart and Scott [16] investigated the carotenoid content of vegetables and fruits commonly consumed in the UK. Leth *et al*. [17] presented the carotenoid contents of Danish food, and Murkovic *et al*. [18] presented an Austrian Carotenoid Database comprising raw vegetables grown in Austria.

In this paper, only data from recent studies on the abovementioned six important carotenoids and their content in foods are reported, covering most of the period from about 2000 to March 2007. Foods included are vegetables, fruits and dairy products, representing the main part of carotenoid intake in Europe. Data about exotic fruits imported into Europe are also included. In Table 1, data on the content of carotenoids in raw and in a few processed foods are presented. Contents refer to the edible part of the food and are stated as μg *per* 100 g fresh weight (or volume). In some papers, contents were related to dry weight and those values were converted to fresh weight and included in Table 1 only when the moisture content of the food was documented. Furthermore, zeaxanthin was sometimes included in the reported lutein content, as the two carotenoids are not separated by all employed analytical methods.

The analytical methods are continuously being improved, leading to more specific data on carotenoids. This also results in data on contents of other carotenoids, *e.g*. phytoene and phytofluene, present in tomatoes and tomato products, and violaxanthin present in other vegetables and fruits, *e.g*. melons. Data for these carotenoids are not included in Table 1.

Several factors affect the composition and content of carotenoids in foods, *e.g*. variety, genotype, season, geographic location/climate, stage of maturity and growing conditions.

2.1.1 Genotype effects

The genotype affects the composition and content of carotenoids in different varieties and cultivars of fruit and vegetables. Lenucci *et al*. [40] showed that the content of lycopene and β-carotene varied significantly among 14 cultivars of cherry tomatoes. Likewise, the total carotenoid content ranged from 3700 to 12 200 μg/100 g among 50 cultivars of red-fleshed watermelons from US [45]. Wall [24] studied composition of different cultivars of banana and papaya. The major carotenoids found in bananas were lutein, α-carotene and β-carotene, and the average content of these carotenoids differed up to two-fold among the two cultivars investigated. Among papaya cultivars, lycopene was found in the red-fleshed samples but not in the yellow-fleshed ones, while β-carotene, β-cryptoxanthin and lutein were present in all samples. In conclusion, there is a high variability in the content of carotenoids in foods reported by different authors.

2.1.2 Seasonal, geographical and cultivation variation

The effects of season, geographic location and cultivation practise on carotenoid composition have been investigated in tomato cultivars. Raffo *et al*. [41] harvested greenhouse cherry tomatoes at full ripeness at six different times of the year. No definite seasonal trend nor correlation with solar radiation or temperature was found for total carotenoids (sum of eight carotenoids), nevertheless tomatoes harvested in mid-summer (July) had the lowest average level of lycopene (7061 μg/100 g), whereas tomatoes from March contained 11 969 μg/100 g. Toor *et al*. [46] also studied sea-

Table 1. Data for the content of major carotenoids in selected foods (μg/100 g or 100 mL fresh weight/volume)

Foods	Lutein	Zeaxanthin	β-Cryptoxanthin	α-Carotene	β-Carotene	Lycopene	Reference
Plant origin							
Apricot	123–188	n.d.–39	–[a)]	n.d.[b)]–44	585–3800	54	[19–21]
Avocado	213–361	8–18	21–32	19–30	48–81	–	[22]
Banana	86–192	–	n.d.–5	60–156	43–131	n.d.–247	[23, 24]
Basil	7050	il[c)]	89	n.d.	4820	n.d.	[18]
Bean, green	883	–	–	–	503	–	[25]
Broccoli	707–3300	il	n.d.	n.d.	291–1750	n.d.	[17, 18, 25]
Cabbage, white	450	il	n.d.	n.d.	410	n.d.	[18]
Carrot	254–510	il	n.d.	2840–4960	4350–8840	n.d.	[17, 18, 26]
Chilli, red	n.d.	–	–	–	6530–15 400	–	[27]
Cornflakes	n.d.–52	102–297	n.d.	n.d.	n.d.	n.d.	[17]
Cress	5610–7540	–	–	–	2720–3690	–	[26, 28]
Cucumber	459–840	il	n.d.	n.d.	112–270	n.d.	[17, 18]
Dill	13 820	il	410	94	5450	n.d.	[18]
Egg plant	170	il	n.d.	n.d.	1110	n.d.	[18]
Endive	2060–6150	–	–	–	1340–4350	–	[26, 28, 29]
Fig	80	–	10	20	40	320	[30]
Grapefruit, red	–	–	–	–	–	750	[20]
Guava	–	–	19–118	n.d.	102–2669	769–1816	[23]
Kale	4800–11 470	–	–	–	1020–7380	–	[31]
Kiwi	–	–	–	–	<20	<10	[32]
Leek	3680	il	n.d.	n.d.	3190	n.d.	[18]
Lettuce	1000–4780	–	–	–	870–2960	–	[25, 26, 28, 29]
Mango	–	–	17–317	n.d.	109–1201	<10–724	[23, 32]
Mandarin juice	–	–	752	n.d.	55	–	[33]
Nectarine, peel	–	–	n.d.–31	–	5–307	–	[34]
Nectarine, flesh	–	–	n.d.–21	–	2–131	–	[34]
Olive oil, extra virgin	350	–	n.d.	n.d.	230	n.d.	[30]
Orange	–	–	74–141	n.d.	171–476	n.d.	[23]
Orange juice	–	–	16–151	n.d.–31	n.d.–98	–	[33]
Papaya	93–318	–	n.d.–1034	n.d.	81–664	n.d.–7564	[23, 24]
Parsley	6400–10 650	il	n.d.	n.d.	4440–4680	n.d.	[17, 18]
Pea	1910	il	n.d.	n.d.	520	n.d.	[18]
Peach	–	–	–	–	–	11	[20]
Peach, peel	–	–	n.d.–36	–	11–379	–	[34]
Peach, flesh	–	–	n.d.–16	–	4–168	–	[34]
Pepper, green	92–911	n.d.–42	n.d.–110	n.d.–139	2–335	n.d.	[18, 25, 26, 35]
Pepper, orange	245	n.d.	3	72	400	–	[35]
Pepper, red	248–8506	593–1350	248–447	n.d.–287	1441–2390	–	[35]
Pepper, yellow	419–638	n.d.	15–41	10–28	42–62	–	[35]
Pineapple		–	70–124	n.d.	139–347	265–605	[23]
Pistachio	770–4900	–	–	–	n.d.–510	–	[36, 37]
Plum, peel	–	–	3–39	–	217–410	–	[34]
Plum, flesh	–	–	3–13	–	40–188	–	[34]
Potato, sweet	50	–	–	–	7830	–	[27]
Pumpkin	630	–	60	–	490	500	[3, 20]
Rhubarb	–	–	–	–	–	120	[20]
Sage	6350	il	87	n.d.	2780	n.d.	[18]
Spinach	5930–7900	il	n.d.	n.d.	3100–4810	n.d.	[18, 38]
Tomato	46–213	il	n.d.	n.d.	320–1500	850–12 700	[17–20, 26, 32, 39]
Tomato, canned	n.d.	n.d.	n.d.	n.d.	217–283	8480–11820	[17]
Tomato, cherry	n.d.–25	–	–	–	300–1100	800–12 000	[32, 40, 41]
Tomato, concentrate	–	–	–	–	–	49 300–94 000	[20]
Tomato juice	29	–	–	–	369	1024–11 000	[20, 42]
Tomato ketchup	n.d.	n.d.	n.d.	n.d.	135–500	4710–23 400	[17, 20, 32, 43]
Tomato puree	n.d.	n.d.	n.d.	n.d.	383–548	13 160–26 110	[17]
Tomato sauce, instant	–	–	–	–	–	5600–39 400	[20]
Tomato soup, instant	–	–	–	–	–	12 400–19 900	[20]
Watermelon, red	–	–	n.d.	n.d.	314–777	4770–13 523	[20, 23]
Watermelon, yellow	–	–	59–110	n.d.	56–287	n.d.–109	[23]

Table 1. Continued

Foods	Lutein	Zeaxanthin	β-Cryptoxanthin	α-Carotene	β-Carotene	Lycopene	Reference
Wheat flour	76–116	n.d.	n.d.	n.d.	n.d.	n.d.	[17]
Wheat flour, durum	164	n.d.	n.d.	n.d.	n.d.	n.d.	[17]
Animal origin							
Butter	15–26	n.d.–2	5–8	n.d.–2	296–431	–	[44]
Cheese, ripened	3	0.2	0.2	n.d.	48	–	[44]
Cheese, young	4	0.2	0.1	n.d.	62	–	[44]
Egg, yolk	384–1320	n.d.	n.d.	n.d.	n.d.	n.d.	[17]
Egg	182	n.d.	n.d.	n.d.	n.d.	n.d.	[17]
Milk, full fat	0.8–1.4	n.d.–0.1	0.3–0.4	n.d.–0.1	15–19	–	[44]
Milk, semiskimmed	0.5–0.8	n.d.–0.1	n.d.–0.1	n.d.	7–9	–	[44]

a) –: not included in the reference(s).
b) n.d.: not detected or quantified.
c) il: included in lutein (stated in the reference).

sonal variations in lycopene content of greenhouse tomatoes, and found lowest contents in the summer months (December–February), as temperatures above 30°C were found to inhibit lycopene synthesis more than the slightly positive effect of solar radiation. Sass-Kiss *et al*. [19] found significantly different contents of lycopene in tomatoes from two successive harvest years due to different weather conditions. In addition, processing varieties of tomatoes grown in open fields contained higher amounts of lycopene than table varieties from greenhouses.

Bergquist *et al*. [38] investigated carotenoids in baby spinach cultivated at three different times within two years. Contents of total carotenoids varied about 15% among the three cultivations at commercial harvest time and about 30% after 5 or 9 days storage at 10°C, while the content tended to increase or remained stable during storage because the metabolic patway of some carotenoids continues during the ripness.

In investigation of Setiawan *et al*. [23], three ripe samples from different regions were analysed, and minimum and maximum values found in *e.g*. mango and papaya were respectively: β-cryptoxanthin 17–317 and n.d.–425 μg/100 g, lycopene 49–724 and 4305–7564 μg/100 g, β-carotene 109–1201 and 322–664 μg/100 g.

Kimura and Rodriguez-Amaya [28] compared hydroponic and conventionally grown lettuce, and found a lower (10–30%) carotenoid content (including lutein and β-carotene) in hydroponic cultivated lettuce.

Caldwell and Britz [47] investigated the effect of supplemental UV radiation on the carotenoid composition of greenhouse leaf lettuce. In general, supplemental UV-B increased the carotenoid content of green leaf lettuce and reduced levels in the red-leaf varieties which may be attributed to light-dependent changes in xanthophilly carotenoids content. Furthermore, up to ten-fold cultivar differences were found in levels of carotenoids in plants grown under identical conditions.

Effects of nitrogen rate and form on the accumulation of carotenoid pigments in the leaf tissue of greenhouse-grown kale were investigated by Kopsell *et al.* [31]. Treatment with different amounts of nitrogen at a constant 1:3 ratio of NH_4–N and NO_3–N showed that concentrations of β-carotene and lutein were not affected by nitrogen rate on a fresh weight basis, however on a dry weight basis the carotenoids increased linearly to increasing nitrogen rate. Increasing NO_3–N from 0 to 100%, at a constant nitrogen rate, resulted in increases in both lutein and β-carotene.

Commercially available Spanish orange juices, including one mandarin juice, were investigated by Melendez-Martinez *et al*. [33]. Hulshof *et al*. [44] found an effect of season on the content of β-carotene in milk samples from the Netherlands. β-Carotene was the predominant carotenoid in all the analysed dairy products even if carotenoid levels in dairy products are extremely low and of very little significance to overall intakes. Milk sampled from January to April contained approximately 20% less β-carotene than milk sampled from July to October, probably due to seasonal differences in animal feeding practices. However, no regional differences as a consequence of homogeneous climate were found.

2.1.3 Stage of maturity and storage

de Azevedo-Meleiro and Rodriguez-Amaya [29] found large differences in the carotenoid contents between young and mature leaves from the same head of endive, lettuce and New Zealand spinach. In endive and lettuce, the carotenoid concentration of the mature leaves were about two to four times those of the young leaves. In contrast, the mature leaves of New Zealand spinach only contained about 75% that of the young leaves, and the principal carotenoids were β-carotene, lutein, violaxanthin, neoxanthin and lactucaxanthin. The coloured compounds in pistachio nuts from different geographical regions (Greece, Iran, Italy, Turkey), each presenting specific varieties, were studied by Bellomo

and Fallico [37]. The level of the main carotenoid, lutein, depended on type of cultivar, cultivation practise and *ripeness* as well as origin of the nuts, the lutein content diminishing with ripening. Among ripe nuts the Italian samples had the highest lutein content.

When storing greenhouse tomatoes at different temperatures for 10 days, Toor and Savage [39], like in earlier observations, found about two-fold more lycopene in tomatoes stored at 15° and 25°C than in refrigerated tomatoes at 7°C (7.5 and 3.2 mg/100 g, respectively).

2.1.4 Potential rich sources

In many developing countries, vitamin A deficiency is widespread, leading to a general need to increase the vitamin A intake of the population, even if the major food source of dietary vitamin A in these area are provitamin A carotenoids. This enhancement of carotenoids might be achieved, *e.g.* by cultivating crops containing higher amounts of provitamin A carotenoids, traditional plant breeding or by genetic engineering [48–50]. Likewise, Western countries focus on fruit and vegetable consumption and the associated health benefits. Carotenoids are among the active components of fruits and vegetables with potential health effects, and enhancement of carotenoid levels might thus be desirable. Examples of investigations into richer sources of carotenoids are outlined below.

Kidmose *et al.* [51] studied carotenoids in different genotypes of spinach. The total carotenoid content varied from 17.76 mg/100 g (in the lightest green genotype) to 22.63 mg/100 g (in the darkest one) with highest β-carotene, lutein and neoxanthin levels. Xu *et al.* [52] analysed the carotenoid composition of peel and juice of ordinary and lycopene-accumulating mutants of orange, pummelo, and grapefruit. Carotenoid profiles of 36 major carotenoids varied with tissue types, citrus species, and mutations. Profiles of peel and juice differed, and content of total carotenoids was much higher in peels.

We summarized the most relevant investigations about the principal food sources of carotenoids. New Zealand spinach are rich in carotenoids, and are one of the most popular leafy vegetables in Brazil and de Azevedo-Meleiro and Rodriguez-Amaya [29] reported levels of about 3800 μg β-carotene, 4800 μg lutein, 2200 μg violaxanthin and 1500 μg neoxanthin *per* 100 g mature leaves. Likewise, Rajyalakshmi *et al.* [53] studied contents of total carotenoid and β-carotene in South Indian forest green leafy vegetables, and found high contents in some varieties. Furtado *et al.* [54] analysed carotenoid content in common Costa Rican vegetables and fruits, and pointed out rich sources. Content of carotenoids in commonly consumed Asian vegetables was studied by Kidmose *et al.* [27]. Many varieties had high contents of β-carotene, lutein and other xanthophylls, *e.g.* drumstick leaves and edible rape turnip leaves. Lako *et al.* [55] reported carotenoid profiles of a wide selection of Fijian fruit and vegetables, and found many rich sources among green leafy vegetables, *e.g.* drumstick leaves as above.

It is also worth noting that the ongoing trend towards globalization is modulating both the availability of foods (*i.e.* exotic fruits, carotenoid-fortified foods), and the social habits in relation to food consumption in some European countries.

2.2 Sampling of foods for carotenoid analysis

In the field of nutrition, sampling is generally aimed at taking samples representative of the eating habits of certain consumers, *e.g.* of the population of a nation. Proper sampling is of utmost importance to avoid unintended variability. When designing the sampling plan for a study of carotenoids in vegetables and fruits, it is important to consider many aspects. Thus a sample plan should include conditions that might influence carotenoid composition and content, *i.e.* cultivation conditions like: choice of variety and cultivar, geographical location, season and year, agricultural practices – like nutrients and fertilizers at disposal, and cultivation in open field or in greenhouse – and stage of maturity. Furthermore, harvesting and postharvest handling, storing, possible processing or cooking, should also be taken into account for a sufficient sample description.

2.3 Analytical methods

Like the above-mentioned agricultural and sampling aspects, the analytical methods by which the carotenoids are determined influence the levels of the different carotenoids.

The general steps in the analyses of carotenoids include: sample preparation, extraction and saponification, separation, detection and quantification. Errors can be introduced in each of these steps.

Several considerations must be taken into account throughout the analysis to get reliable results, as carotenoids are highly susceptible to isomerization or degradation from light, heat, oxygen, acids, prooxidant metals and active surfaces [56–58]. Otherwise, the carotenoids might to some extent undergo isomerization or degradation.

2.3.1 Sample preparation

Before homogenization, an appropriate portion of the food, *e.g.* vegetables should be trimmed and cleaned and only those parts that are normally eaten should be included in the analyses [18]. The foods might be lyophilized or frozen to avoid changes in the carotenoid concentrations before preparation. These procedures should ensure that representative samples are ready for extraction.

2.3.2 Extraction and saponification

In food analyses, the procedure normally includes extraction of the carotenoids followed by alkaline saponification

of the ester forms present in certain foods. In addition, the saponification step removes interfering substances like chlorophylls and unwanted lipids before the final extraction of the carotenoids. Saponification is not necessary for samples without these compounds.

Several extraction procedures have been applied, and have been described in other reviews [57–60]. Numerous organic solvents have been used either alone or in mixtures for liquid-liquid extraction, which is the general procedure. As an alternative to the traditional method, supercritical fluid extraction (SFE) has been applied in some recent investigations [61]. To prevent carotenoid losses during extraction, antioxidants such as butylated hydroxytoluene (BHT) are usually added to the extraction solvent. Moreover, internal standards might be used to assess losses during the extraction [15, 62]. In some studies, an SPE is added as a further purification of carotenoids prior to the determination [17].

2.3.3 Separation, detection and quantification

Traditionally, determination of carotenoids in foods was performed by measuring the total absorption of the extract at a specific wavelength and calculating the amount using β-carotene as standard. This was later improved by separation of carotenes and xanthophylls by open-column chromatography (OCC). The introduction of HPLC equipped with UV and/or PDA detectors made the isolation, detection and quantification of the individual carotenoids possible, thus greatly enhancing the quality of the analytical results. More recently, the application of HPLC coupled with MS (LC-MS) has proven a powerful tool for identification of carotenoids. This technique is very sensitive and might also provide information about structure. By coupling HPLC with NMR the structure of the carotenoids might be completely elucidated.

There are no general HPLC conditions of choice neither for mobile phase nor column [30, 57, 60]. Both normal-phase and RP HPLC can be applied to separate the carotenoids [19, 63, 64]. However, the most frequently used systems are RP [59]. Many different solvents have been applied as gradient or isocratic mobile phases. To prevent oxidation of carotenoids, an antioxidant is often added to the mobile phase. The column selection depends on the requirements for the separation of the individual carotenoids and their isomers. Monomeric C18 columns separate most of the xanthophylls, but not lutein and zeaxanthin, whereas these components can be resolved with polymeric C18 columns [65]. Similarly, the nonpolar carotenoids, *e.g.* α- and β-carotene, are poorly resolved with the monomeric C18 columns and partly separated with the polymeric C18 columns. Since Sander and Wise [65] showed an improved separation of both polar and nonpolar carotenoids including geometric isomers with a polymeric C30 column, this type of column has been used for a variety of food analyses [19, 66, 67].

2.3.4 Quality assurance and standard methods

To get reliable results in analysis of carotenoids it is always advisable to include measures of quality assurance. Preferably, the method should be validated and, *e.g.* sensitivity, selectivity, recovery, repeatability and reproducibility estimated. Scott *et al*. [56] developed a vegetable mix reference material (RM), and the use of standard or in-house RMs is highly recommendable [18] for assuring the analytical quality. Furthermore, purity of the carotenoids should be considered and care taken in the standardization of carotenoid solutions [16].

As reported above, no generally applicable standard method for determination of individual carotenoids in food has been introduced. However, standard methods are available from the Association of Analytical Communities (AOAC) [68] using OCC with spectrophotometric determination of carotenes and xanthophylls, respectively and European Committee for Standardization (CEN) [69] has published a standard method for determination of total β-carotene by HPLC with UV–Vis detection.

2.4 Carotenoid intake

It is widely assumed that serum concentrations of carotenoids reflect, at least to some extent, the consumption of carotenoid-containing foods [70]. The influence of diet as a factor of serum carotenoid concentrations has long been known, although both dietary intake and serum concentrations of carotenoids have shown a high variability both within and between subjects in different populations [71–75]. Seasonal variations in individual carotenoid intake, and serum concentrations, have been reported in some European countries (*i.e.* Spain) while not in others (*i.e.* UK, Republic of Ireland, Finland) [3, 73, 74, 76], even when total carotenoid intake may not vary significantly (*i.e.* Spain) [3]. Although fluctuations between seasons may be observed for several carotenoids both in the diet and serum levels [74–77], in Spanish diet, these reach statistical significance only for β-cryptoxanthin (higher in winter) and lycopene (higher in summer); these changes are found to be in accordance with the availability and consumption of the major dietary contributors (*i.e.* citrus fruits and tomato and watermelon, respectively) [76, 77].

A European north–south gradient for the intake of some carotenoids and serum concentrations, both within and between European countries, have been reported [3, 75, 78, 79]. This pattern is consistent with food availability data (*i.e.* fruits and vegetables) among European countries since southern (Mediterranean) countries (*i.e.* Greece, Italy, Portugal, Spain) consume greater amounts of fruits and vegetables than northern countries (*i.e.* UK, Ireland, Scandinavian countries) [80, 81]. In some countries, this geographical trend has been reported for both total and individual carotenoid intake and, overall, it is associated with variations in fruit and vegetables consumption (*i.e.* in UK, low in the

North) and with socioeconomic status and cultural factors. In fact, the specific traditional and cultural factors between the two groups of populations, and in addition the changes in marketing could contribute to the change of life style [78]. Consistently, serum levels also show this distribution trend across north–south axis.

Time trends in carotenoid intake have been scarcely assessed in European countries. Nonetheless, changes in major dietary sources of carotenoids (fruits, vegetables, cereals and recently fortified foods) is known to have occurred in European countries during the last decades [81–83] which is partly explained by changes in socio-economical, demographic and cultural factors. Time variation, on a short-term basis, in carotenoid intake has been assessed in Denmark, where, apparently, intake pattern of carotenoids has not changed from 1995 to 1997 [17]. Similarly, in Spain, using almost the same methodology, a fairly consistent qualitative and quantitative pattern of carotenoid intake from fresh fruits and vegetables was observed on a short-term basis, *i.e.* between 1996 and 2004, although this pattern was different when data were calculated on a longer time scale, *i.e.* 1960–1980 (it could be due to changes in fruit and vegetables consumption of populations) [77].

2.5 Methodology

Estimated intakes of carotenoids vary widely both on an individual, regional and national level, and significant seasonal variations in intake of individual carotenoids have been also reported in some countries (*i.e.* Spain) [76, 77]. Carotenoid intake assessment, at both the individual and group level, has been shown to be complicated mainly for the high variability within-subject and between-subject intake, inaccuracies associated with methods of dietary assessment, and inconsistencies in food composition tables (FCTs) and databases [84–86].

The food balance sheets (FBS) [83] present a comprehensive picture of the pattern of a country's food supply during a specified reference period. The FBS shows for each food item – *i.e.* each primary commodity and a number of processed commodities potentially available for human consumption – the sources of supply and its utilization. The total quantity of foodstuffs produced in a country added to the total quantity imported and adjusted to any change in stocks that may have occurred since the beginning of the reference period gives the supply available during that period. On the utilization side a distinction is made between the quantities exported, fed to livestock, used for seed, put to manufacture for food use and nonfood uses, losses during storage and transportation, and food supplies available for human consumption. The *per capita* supply of each such food item available for human consumption is then obtained dividing the respective quantity by the related data on the population actually partaking of it. Data on *per capita* food supplies are expressed in terms of quantity and – by applying appropriate food composition factors for all primary and processed products – also in terms of caloric value and protein and fat content.

Table 2. Sources of nutritional data

Level	Source	Type of data
Population	Food balance sheets	Ecological; large units
Household	House budget survey	Ecological; small units
Individual	Nutrition survey	Analytical; individuals

Source: EURONUT, Report 9, 1987 [87].

Table 3. Availability of data for lutein and/plus zeaxanthin content in foods nutritional and epidemiological studies

Ref.	Type of report	Country (food origin)	Lutein	Lutein + Zeaxanthin
[14]	HPLC report	USA	Yes	
[89]	HPLC report	Finland		Yes
[90]	HPLC report	Malaysia	Yes	
[91]	HPLC report	Spain	Yes	
[92]	Database	World wide		Yes
[2]	Database	USA (several)		Yes
[16]	HPLC report	UK	Yes	
[93]	Database	Spain	Yes	
[94]	HPLC report	USA	Yes	
[15][a)]	Database	USA (USA)		Yes
[18]	Database	Austria		Yes
[3]	Database	Europe (several)		Yes

a) Zeaxanthin values reported independently for selected foods.

Source: Permission *Brit. J. Nutr.*: Granado *et al.* 2003 [88].

Carotenoids content has been calculated applying USDA FCTs.

Sources of nutritional data have been classified at different levels and data obtained are of different type (Table 2) [87].

Regardless of the confidence in the method used for dietary assessment, evaluation of nutrient exposure by dietary means is based on the availability of reliable food composition data. Since the nutritional interest in carotenoids was largely due to their provitamin A activity, traditionally, FCTs and databases have, traditionally, not included values for individual carotenoids in foods, although they have considered vitamin A (retinol equivalents) content. However, the increasing evidence of the potential role of several constituents present in fruits and vegetables (carotenoids) in human health led to a revision of former data and the inclusion of nonprovitamin A carotenoids (*i.e.* lutein) in the new FCTs and databases during the 1990s (Table 3) [88].

2.6 Available data of dietary intake

Few studies have been carried out to ascertain the total intakes of carotenoids in the European diet. A European

Table 4. Comparison of carotenoid intake (mg/day) in adults in five European countries (data are medians and interquartile ranges)

	β-Carotene		Lutein (+ Zeaxanthin)		Lycopene		α-Carotene		β-Cryptoxanthin		Total carotenoids	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
Spain (n70)	2.96	1.58–4.41	3.25	1.75–4.34	1.64	0.50–2.64	0.29	0.15–0.51	1.36	0.74–2.16	9.54	7.16–14.46
France (n76)	5.84	3.83–8.00	2.50	1.71–3.91	4.75	2.14–8.31	0.74	0.37–1.36	0.45	0.17–0.88	16.06	10.3–22.1
UK (n71)	5.55	3.66–6.56	1.59	1.19–2.37	5.01	3.2–7.28	1.04	0.71–1.66	0.99	0.32–1.64	14.38	11.77–19.1
Rep of Ireland (n76)	5.16	3.47–7.42	1.56	1.14–2.1	4.43	2.73–7.13	1.23	0.69–1.78	0.78	0.4–1.44	14.53	10.37–18.9
The Netherlands (n75)	4.35	2.93–5.7	2.01	1.42–3.04	4.86	2.79–7.53	0.68	0.30–0.90	0.97	0.50–1.75	13.71	9.98–17.7

Source: Permission *Brit. J. Nutr.*: O'Neill *et al.* 2001 [3].

Table 5. Intake (mg/person/day) reported in several European countries

Ref.	Lutein (+ zeaxanthin)	β-Cryptoxanthin	Lycopene	α-Carotenoid	β-Carotenoid	Dietary method/database	Foods/population assessed (subjects)
[73]	0.92	–	1.03	–	2.21	4 days collection HPLC data	Vegetables UK; *N* = 79
[95]	0.67	0.14	0.74	–	1.51	7 days diary carotenoid database	Total diet UK (EPIC Norfolk cohort) N = 176 controls
[78]	–	0.022–0.033	–	0.31–0.34	1.47–1.70	4 days weighed records (+ eating out)	Total diet UK (National Diet and Nutrition Survey, *N* = 1.478 (<65 years)
[96]	2.45/2.55 (w/m)	0.21/0.16 (w/m)	1.30/1.05 (w/m)	0.69/0.69 (w/m)	2.90/2.96 (w/m)	Dietary questionnaire; energy-adjusted intake Harvard School of Public Health database	Total diet The Netherlands *N* = 120.693[a]
[96]	1.15	0.03	0.65	0.53	1.76	Dietary questionnaire; energy-adjusted intake Harvard School of Public Health database	Total diet Finland (ATBC study, placebo branch); *N* = 6.771 men
[76]	0.58	0.41	1.25	0.22	1.00	Family Budget Survey HPLC data	Fresh fruits and vegetables Spain; *N* = 72.279
[97]	–	–	–	–	3.1–5.0	Two 24 h recalls; CIQUAL database	Total diet Spain; *N* = 2.346
[98]	1.47	–	0.95	0.24	2.11	Dietary history questionnaire EPIC database (2nd Edn.)	Total diet Spain; *N* = 354[b]
[99]	0.90	0.64	2.09	0.26	1.99	Dietary history questionnaire EPIC database (2nd Edn.)	Total diet Spain (EPIC cohort), *N* = 41.446
[77]	0.45	0.31	1.16	0.26	1.07	Family Budget Survey HPLC data	Fresh fruits and vegetables Spain; *N* = 6.000 households
[100]	4.01	0.17	7.38	0.15	2.6	Seven-day dietary diary HPLC data	Total diet Italy (INN-CA Study); *N* = 1.968

a) Netherlands Cohort study; 62.412 men, 58.279 women (assessed at baseline).
b) Subjects considered as controls were patients admitted to the hospitals with a variety of diagnosis unrelated to the principal study factors (gastric cancer).

carotenoid food database was published along with the assessment, by a Food Frequency Questionnaire (FFQ) at individual level, of the carotenoid intakes of people groups in a five-country comparative study [3]. Main results are presented in Table 4. However, it should be noticed that the population used in this study was a group in a determined area of each of the five participant countries (*ca*. 80 subjects *per* country). When interpreting the data provided by that study, it should be considered that the levels of intake reported in this study are somewhat consistent with the findings in serum of the same individuals. That is, the relative crude intake and the relative contribution of xanthophylls and carotenes indicate 'true' differences in carotenoid intake (and food sources) among European countries. Participants may not necessarily be representative of the overall population although it was assumed that they followed a typical food intake pattern characteristic of their country. In addition, all subjects filled out a common FFQ.

Table 5 summarizes carotenoids intake in some European countries (UK, Finland, The Netherlands, Spain and Italy) from representative literature. To have an overall view of other countries, we have to take into account the analysis of FBSs. However, both crude data and comparisons should be considered with caution since, as shown, sample size and methodology differ between studies.

Table 6 shows the percentage contribution of individual food items evaluated by FFQ to the total intake of each of the five carotenoids in parentheses.

Table 6. Major foods contributing to carotenoid intake in adults in five European countries

	France (Grenoble)	Republic of Ireland (Cork)	UK (Coleraine)	The Netherlands (Zeist)	Spain (Madrid)
	Name (%)	Name (%)	Name (%)	Name (%)	Name (%)
β-Carotene	Carrots (38)	Carrots (60)	Carrots (53)	Carrots (42)	Spinach (26)
	Spinach (14)	Tomat. prod (13)	Soups (10)	Spinach (12)	Carrots (24)
Lutein	Spinach (31)	Peas (19)	Peas (36)	Spinach (30)	Spinach (34)
	Lettuce (8)	Broccoli (16)	Broccoli (8)	Broccoli (10)	Lettuce (16)
	Eggs (8)	Eggs (10)	Eggs (8)	Peas (9)	Oranges (7)
	Mix vegetables (6)	Carrots (9)	Sweetcorn (7)	Chicory (8)	Eggs (7)
Lycopene	Tomatoes (25)	Tomatoes canned (23)	Tomatoes (21)	Tomato soup (29)	Tomatoes (55)
	Tomatoes canned (16)	Tomato soup (17)	Tomatoes canned (20)	Tomatoes (16)	Tomato puree (42)
	Pizza (16)	Pizza (16)	Pizza (15)	Pizza (16)	
α-Carotene	Carrots (82)	Carrots (90)	Carrots (88)	Carrots (87)	Carrots (60)
	Oranges (6)	Coleslaw (5)	Coleslaw (6)	Oranges (5)	Tangerines (17)
β-Cryptoxanthin	Orange juice (50)	Oranges (42)	Orange juice (45)	Tangerines (41)	Tangerines (53)
	Oranges (30)	Tangerines (28)	Oranges (26)	Orange juice (33)	Oranges (38)

Source: Permission *Brit. J. Nutr.*: O'Neill *et al.* 2001 [3].

Table 7. Ten top contributors (%) to lutein (+ zeaxanthin) intake in five European countries[a)]

France ($N = 76$)	Republic of Ireland ($N = 76$)	UK ($N = 71$)	The Netherlands ($N = 75$)	Spain ($N = 70$)
Spinach (31)	Peas (19)	Peas (36)	Spinach (30)	Spinach (34)
Lettuce (8)	Broccoli (16)	Broccoli (8)	Broccoli (10)	Lettuce (16)
Eggs (8)	Eggs (10)	Eggs (8)	Peas (9)	Oranges (7)
Mix vegetables (6)	Carrots (9)	Sweetcorn (7)	Chicory (8)	Eggs (7)
Cucumber (6)	Tomato (8)	Lettuce (6)	Lettuce (4)	Broccoli (6)
Green beans (4)	Oranges (7)	Carrots (4)	Tomato (4)	Peas (6)
Courgette (4)	Peppers (6)	Tomato (4)	Oranges (4)	Potatoes (3)
Peas (3)	Sweetcorn (4)	Tangerines (4)	Eggs (4)	Tangerines (3)
Tomato (3)	Spinach (3)	Celery (4)	Green beans (4)	Peppers (3)
Sweetcorn (2)	Lettuce (3)	Spinach (3)	Potatoes (4)	Leeks (2)
Total (%) 75	*Total (%) 85*	*Total (%) 84*	*Total (%) 81*	*Total (%) 97*
Green veg. 56%	Green veg. 47%	Green veg. 57%	Green veg. 65%	Green veg. 67%

a) Assessed in winter.
Source: Permission *Brit. J. Nutr.*: Granado *et al.* 2003 [88].

Table 7 shows estimations using data obtained in a European multicentre study where dietary intake was estimated using a common FFQ and database of carotenoids in food [88].

As shown, although green vegetables are important contributors to lutein intake in five European groups, relative contribution differs substantially among them. It is also worth noting the relative contribution of nongreen vegetables and fruits and the fact that nongreen foods may account for almost half of the total lutein intake in some groups. More importantly, zeaxanthin, is mostly provided by nongreen vegetables and fruits [76, 88].

In Table 8, a comparison between countries on the relative contribution of each carotenoid to total carotenoids intake calculated from FBS is reported [4, 83].

While several methodological constraints (databases, groups assessed and method for dietary assessment) limit the comparability of crude intakes of carotenoids among groups, an alternative approach to compare groups/populations is to estimate the relative contribution of each carotenoid to the total intake. This approach does not overcome all the constraints regarding the reliability of the data used for comparison but may provide an interesting picture for comparative (ecological) purposes. This approach is based on several facts:

(i) The relative contribution of each carotenoid has some association with its crude intake (g/person/day), and therefore the intake of its major dietary sources, and provides information for each carotenoid (and food sources) within the context of the total diet. For example, intake of β-cryptoxanthin may be similar in two groups but the contribution to total carotenoid intake may be significantly different.

(ii) The above point relates to other nutritional and physiological facts. Carotenoids may interact with each other (synergistic and antagonistic) during absorption, transport, deposition and biological action. Thus, the relative amount of each class and type of carotenoid in the total diet become relevant [101].

Table 8. Relative contribution (%) of each carotenoid intake to total carotenoid intake according to FBSs data [83].

Country	Total intake μg/day[a)]	Lutein (+ zeaxanthin)	β-Cryptoxanthin	Lycopene	α-Carotene	β-Carotene
Germany	9.368	52	3	8	3	33
Denmark	10.092	52	4	7	3	34
Italy	15.753	45	4	15	3	33
Sweden	7.521	48	5	11	3	32
UK	8.654	50	4	9	3	33
Greece	20.968	40	3	21	4	32
France	13.984	50	4	9	3	34
The Netherlands	8.761	48	5	10	3	33
Spain	12.789	45	4	14	3	34
Europe	*11.786*	*48*	*4*	*12*	*3*	*33*

a) Sum of lutein (zeaxanthin), β-cryptoxanthin, lycopene, α-carotene and β-carotene.
Based on data from USDA Food Composition Tables [4].

Table 9. Relative contribution (%) of each carotenoid intake to total carotenoid intake[a)]

Country (ref.)	Carotenoid intake (mg/person/day)[a)]	Foods assessed	Lutein (+ zeaxanthin)	β-Crypto-xanthin	Lycopene	α-Carotene	β-Carotene
Spain							
[76]	3.5	Fresh fruits and vegetables	17	12	36	6	29
[3]	9.54	Total diet	37	14	17	3	31
[99]	5.88	Total diet	15	11	36	4	34
[102]	3.25	Fresh fruits and vegetables	14	10	36	8	33
The Netherlands							
[103]	6.1						21
[3]	13.71	Total diet	15	7	35	7	32
[96]	7.55	Total diet	32 (M)	3	17	9	38
	7.41		34 (W)	2	14	9	40
Finland							
[104]	4.0	Total diet	28	<1	20	3	50
[96]	4.12	Total diet	28	<1	16	13	43
France							
[3]	16.06	Total diet	16	3	30	5	36
UK							
[3]	14.38	Total diet	11	7	35	7	39
Rep. Ireland							
[3]	14.53	Total diet	11	5	30	8	36
Overall range (%)	–		*11–37*	*0–14*	*14–36*	*3–13*	*21–50*

a) Mean or median values; total carotenoid intake = sum of lutein, zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene.

(iii) Finally, because of each carotenoid may display different biological functions, actions and associations, relevant both at individual and population level, the relative occurrence of each carotenoid within the total diet may become important when comparing groups within an epidemiological context.

For example, as shown in Table 9 based on the data reported by O'Neill *et al*. [3], using the same dietary method and database, α-carotene and β-carotene show a consistent contribution in the European countries (3–9 and 31–39%, respectively), regardless of the dietary habits and geographical origin of the groups assessed. On the contrary, for lutein and lycopene, a different contribution pattern is observed between Spain (37 and 17%, respectively) and the rest of the European countries (11–16 and 30–35%, respectively). Regarding β-cryptoxanthin, a clearly distinct relevance is observed with Spain showing two- to three-fold more contribution than in others, especially north European countries. All these values are consistently below the mean/median values reported, for example, in Spain (0.3–0.6 mg/day) [3, 76,

77]. Thus, regardless of the method of dietary assessment and used database, sample size and endpoint measured, it is interesting to note that, compared to other dietary carotenoids, β-cryptoxanthin contribute marginally (0–7%) in North European countries (Finland, Denmark, Germany, England, Ireland), whereas in the south (*i.e.* Spain) it accounts for 10–14% (annually) and up to 20% (*i.e.* winter) to total carotenoid intake [3, 76, 77]. While this approach seems to be useful to compare exposure (nutrient intake) in different groups, it also depends on the method of assessment. This fact is highlighted when this is approached using FBSs as shown in Table 8. The apparent lack of variation in the relative contribution among European countries (except for lycopene and lutein) contrasts with the figures obtained in the individual studies performed in the same countries. This may be apparently due to the method used to estimate dietary intake since FBS provide figures on food availability (not consumption) while the individual studies provide information about 'true' nutrient intakes of the individuals although by different methods.

3 Effects of food processing on carotenoid stability and/on bioavailability

Carotenoid content and pattern of food material are modified during postharvest storage of plant materials, as well as during processing – at home or industry – and storage of food products. Particularly, thermal processing (*i.e.* blanching, pasteurization, cooking, canning, frying and drying) may decrease carotenoid contents, but at the same time may be beneficial through the disruption of food matrices (*e.g.* cell walls and membranes) and so facilitating the liberation (bound) and solubilization of carotenoids (free and ester forms) resulting in an increased carotenoid bioavailability. Processing operations that reduce the particle size of food material (*e.g.* chopping, grinding, milling or homogenation) or the incorporation of an oil-phase in food formulations (*e.g.* addition of oil to salads, emulsioning), may also enhance carotenoid bioaccessibility [105–109]. Emerging technologies (*e.g.* high pressure-low temperature, pulse electric fields) and several new approaches in food packaging (*e.g.* modified atmospheres, addition of antioxidants and active packages) in addition may modify carotenoid contents of food [110, 111]. Therefore, food processing implies a relevant impact on the nutritional quality of food and the stability of micronutrients in foods during food supply. Thus, food processing has a relevant impact on the dietary patterns of the population.

3.1 Postharvest storage

Mayer-Miebach and Spieß [112] reported that the total carotenoid content of *Kintoki* carrots was reduced by about 30% of the initial amount during 8 wks of storage at 1°C with 97% humidity. Lycopene content was reduced to about 60%, while only 20% of the β-carotene content was lost.

Kopas-Lane and Warthesen [113] found that the lutein content in spinach was nearly stable during storage at 4°C for 8 days in the dark, whereas up to 22% was lost when exposed to light.

3.2 Thermal processing

The scientific literature shows a wide variability of effects depending on the time/temperature conditions used (Table 10). The effects of important unit operations often used in industry are described below.

3.2.1 Kinetics of thermal degradation/isomerization

Studies towards the kinetics of thermal degradation and isomerization of carotenoids in food matrices are scarcely found in literature. Dewanto *et al.* [146] showed that the amount of all-trans-lycopene extracted from tomato homogenates, subjected to heat treatment at 88°C increased significantly 1.6-fold after 2 min and 2.7-fold after 15 or 30 min, as compared to non heated homogenates. The total (*Z*)-lycopene content increased by 6, 17 and 35% after 2, 15 and 30 min, respectively. After subjecting *Nutri Red* carrot purees with a 1% oil supplement to 2 h heat treatments at 100, 110, 120, 130 and 140°C, (all-*E*)-lycopene content decreased to 60, 63, 63, 38 and 25% of the initial value, respectively. Oil supplements had no effect on (all-*E*)-lycopene but slightly reduced isomerization. In samples without oil, (9*Z*)-lycopene increased by 10-, 30-, 41-, 43- and 38-fold at 110, 120, 130 and 140°C, respectively. Heat treatment at 70°C degraded only slight amounts of (all-*E*)-lycopene even after a 5 h heating time [121]. In homogenates of a zeaxanthin and lutein containing potato variety, the treatment temperature (25–150°C) had a much more marked effect on the carotenoid pattern than treatment time (0–5 h). The potato variety used for all experiments contained several carotenoids, mainly zeaxanthin (0.2–0.8 mg/100 g) and lutein (0.04–0.16 mg/100 g) [147]. At temperatures above 70°C lutein was totally degraded, while zeaxanthin was stable even for high-temperature and long-time treatments, regenerating 9-*cis*-zeaxanthin.

3.2.2 Blanching/pasteurization

Blanching (70–105°C) and pasteurization (60–85°C) are mild heat treatments for short time periods used to inactivate enzymes and vegetative microorganisms. Data obtained by Aman *et al.* [114], analysing spinach after steam-blanching for 2 min, have shown a decrease of total lutein (17%) and (9*Z*)-lutein contents (7%), while the (13*Z*)-isomer level was unaffected. According to Choe *et al.* [115], the lutein content of spinach was stable during blanching and steaming for 2 and 5 min, respectively. Control samples contained 30.99 mg lutein and 42.86 mg β-car-

Table 10. Effect of thermal processing on stability of some nonprovitamin A carotenoids

Technology	Product	Bioactive compound	Effect	Ref.
Blanching	Carrot (*Kintoki*)	(all-*E*)-lycopene	–	[112]
	Spinach	(all-*E*)-, (13*Z*)-lutein	–	[114, 115]
		(all-*E*)-, (9*Z*)-lutein	↓	[114, 115]
Pasteurization	Tomato (puree)	(all-*E*)-lycopene, -lutein	–	[116]
	Orange (juice)	(all-*E*)-lutein	↑	[117]
		(all-*E*)-zeaxanthin	–	[117]
	Orange – carrot (juice mix)	(all-*E*)-lutein	–	[118]
		(all-*E*)-zeaxanthin	↑	[118]
Cooking	Tomato (homogenates)	(all-*E*)-, *cis*-lycopene	↑	[112]
	Tomato (juice)	Total lycopene	–	[119]
		cis-Lycopene	↑	[119]
	Tomato (pulp)	(all-*E*)-lycopene	↓	[120]
	Carrot (*Nutri Red*) with/without oil	(all-*E*)-lycopene (9*Z*)-lycopene	↓↑	[121]
	Broccoli, spinach, green beans	(all-*E*)-lutein	↑	[122]
Canning	Tomato (pulp)	(all-*E*)-lycopene	↑	[123]
	Carrot (*Nutri Red*) with/without oil	(all-*E*)-lycopene (9*Z*) lycopene	↓↑	[121]
	Kale, corn, spinach, green peas	Total lutein, zeaxanthin	↑	[124]
	Corn	(all-*E*)-lutein, -zeaxanthin	–	[125]
	Sweet corn	(all-*E*)-lutein, -zeaxanthin	↓	[114]
Osmotic treatment	Carrot (*Nutri Red*)	(all-*E*)-lycopene	↑	[126]
Hot air drying	Tomato, carrot (*Nutri Red*)	(all-*E*)-lycopene	↑	[121, 127, 128]
	Tomato	(all-*E*)-lycopene	–	[129, 130]
	Tomato (paste)	(all-*E*)-lycopene	↓	[131]
	Tomato	(all-*E*)-lutein	↑	[129, 130, 132]
	Potatoes	(all-*E*)-zeaxanthin	↑	[133]
	Red pepper (whole/cut pods)	(all-*E*)-zeaxanthin	↑	[134, 135]
	Pepper (whole pods), Paprika	(all-*E*)-zeaxanthin	↓	[136, 137]
Frying	Potatoes	(all-*E*)-lutein	↑	[138]
	Carrot (chips)	Total carotenoids	↓	[139]
Microwave heating	Carrot (*Nutri Red*) (slices)	(all-*E*)-lycopene	–	[12]
	Broccoli	(all-*E*)-lutein	↑	[124, 140]
	Spinach, green beans, broccoli	(all-*E*)-lutein	–	[122]
	Sweet potatoes (leaves)	(all-*E*)-lutein	↓	[141]
	Papaya, broccoli (florets)	Total carotenoids	↓	[140, 142]
Multistep heat-treated products	Tomato (various commercial products)	(all-*E*)-, *cis*-lycopene	–	[119]
	Tomato (paste)	(all-*E*)-lycopene	↑	[143]
			↓	[144]
		cis-Lycopene	–	[143]
		(all-*E*)-lutein	–	[143]
	Orange (juice)	Total carotenoids	↓	[145]

–, No changes; ↑, increase; ↓, decrease.

otene *per* 100 g sample. In an orange – carrot juice mixture, no variations in lutein content were observed after pasteurizing at 98°C for 21s, while about 45% more zeaxanthin was detected due to an enhanced extractability [118]. The same effect was shown after blanching of lycopene containing *Kintoki* carrots at 90°C for 15 min, which raised lycopene content for about 15% [107]. In tomato puree, lycopene and lutein contents were not affected by pasteurization [116].

3.2.3 Cooking/canning

A prolonged heating time of 2 h at 100°C caused a partial decrease (18%) of lycopene content in tomato pulp; (*Z*)-isomers were not detected [120]. The amount of lutein extracted from green peas increased by about 10 – 15% after boiling for 1 h [122]. In sweet corn, canning at 121°C in a rotary retort decreased total lutein and zeaxanthin content by 26 and 29%, respectively, while the amounts of (*Z*)-lutein and (*Z*)-zeaxanthin increased from 12 to 30% and from 7 to 25%, respectively. (13*Z*)-isomers of both lutein and zeaxanthin prevailed as individual stereoisomers [143]. So, from examples above mentioned, the different way of cooking could lead to a decreasing, an increasing or no variations in the content of single carotenoids; in addition, the way of cooking could modify the profile of carotenoid content in relationship with food matrix and stability of specific carotenoids in the foods.

3.2.4 Multistep heat treatment

After a commercial hot-break extraction of tomato paste at 90°C for 5 – 10 min followed by concentration under vacuum at 60 – 70°C and final sterilization at 121°C for

30 min, the amount of (all-*E*)-lycopene extracted was enhanced about 1.4-fold, while the (*Z*)-lycopene and lutein contents remained unchanged [115]. Also, results obtained by Agarwal *et al*. [119] indicated the stability of (all-*E*)-lycopene under industrial processing conditions: raw tomatoes and various commercial tomato products, after a multi-step heat-treatment, were found to contain 5–10% (*Z*)- and 90–95% (all-*E*)-lycopene; no difference was observed.

3.2.5 Drying

For hot air drying, whole or chopped plant material is generally exposed to temperatures not exceeding 80°C. Therefore, no significant carotenoid losses or generation of (*Z*)-isomers are expected. However, oxidative losses may occur in some traditional slow drying methods that last over a period of few days. Much higher inlet temperatures are used for spray-drying, thus raising the probability of (*Z*)-isomer generation. Goula and Adamopoulos [131] observed oxidative lycopene losses (up to 32%) during spray-drying (air inlet temperature: 110–140°C) of tomato paste. On the other side, no significant carotenoid losses were observed in tomatoes dried at lower temperature (42°C) [130]. Enhanced carotenoid extractability after hot air drying has been reported by various authors: lycopene [121, 127, 128], lutein [132], zeaxanthin [133].

3.2.6 Frying

For frying, the material is cut, blanched, sometimes soaked in an antioxidant solution and, finally fried in fat or oil pre-heated to temperatures of 150–180°C. Food material is heated rapidly in the surface layers to the temperatures of the frying medium; however temperature does not exceed 100°C in inner layers. Lutein remained stable after frying of eight different potato varieties and a higher extractability of lutein was reported [138].

3.2.7 Microwave heating

The main industrial applications of microwave heating are tempering, baking and drying; other uses include blanching and cooking. In papaya, microwave blanching induced small losses of the total carotenoids [142]. Khachik *et al*. [122] studied the effect of microwave cooking on lutein retention and its (*Z*)/(*E*) ratio for different vegetables. Under mild cooking conditions (750 W; spinach: 1.5 min; green beans: 4 min; broccoli: 5 min), the lutein levels and (*Z*)/(*E*) ratios remained unchanged. During microwave cooking (700 W) of sweet potato leaves, (all-*E*)-lutein losses increased with increasing cooking times of up to 56% after 8 min; no (*Z*)-lutein isomers were formed. The (9*Z*)-lutein, contained in the fresh leaves, was completely degraded, and two lutein dehydration products were identified [141]. After microwave vacuum drying with a microwave power program of 400 W continuously, (all-*E*)-lycopene content remained stable in *Nutri Red* carrot slices. However, significant losses of carotenoids were observed, when a combined microwave power programme (600/240 W), by which high temperatures were generated, was used. No (*Z*)/(*E*) isomerization took place [121].

3.3 Product storage

The effects of food storage are summarized in Table 11.

3.3.1 Frozen storage

Long term frozen storage has been found to cause a reduction of the carotenoid content. For example, for watermelons, a decrease of up to 40% of the lycopene content was observed after 1-year storage at temperature ranges between −20 and −80°C [148]. However, lycopene was stable for three months in diced tomatoes stored at −20 and −30°C [149]. The exclusion of oxygen during frozen storage of tomato products reduces the rate of lycopene degradation [150, 151]. During frozen storage of pizza, the rate of degradation of the lycopene contained in the tomato ingredient is much faster than during frozen storage of the ingredient (tomato dices or purees) [150]. Depending on the packaging method (with/without oxygen exclusion; with/without paper box) up to 70% of lycopene may be destroyed.

3.3.2 Cold storage

The cold storage of minimally processed (MP) plant material – generally freshly cut and washed – has been studied by several authors. de Azevedo-Meleiro and Rodriguez-Amaya [29] reported a 19% reduction of the lutein content of MP endive after 5 days storage at 7–9°C. The lycopene content of MP watermelon (75% of the total carotenoids) slightly decreased during storage at 9°C, however stored at 5°C under light the lycopene losses were lower [34].

3.3.3 Storage at room temperature

During 1-year storage of commercially canned tomato juice no significant lycopene loss was observed at 25°C either at 37°C [119]. In commercially prepared tomato pulp, puree and paste lycopene remains stable even when stored under conditions of accelerated aging at 30, 40 and 50°C up to 90 days [154]. Light has an effect on the isomerization of lycopene in tomato juice: after 12 wks storage at 25°C in the dark the formation of (9*Z*)- and (13*Z*)-lycopene was favoured, while after the same time at the same temperature but using light storage (13*Z*) and (15*Z*) were the predominant lycopene isomers [159].

During storage of dried tomato products oxidation and isomerization are the main mechanisms of (all-*E*)-lycopene loss. In powders, with a great specific surface exposed to the storage conditions, an increased sensitivity for oxidative lycopene losses can be expected. Isomerization increases with increasing storage time and under illumination conditions; however oxidation increases mainly due to increased storage temperature. The residual moisture of the product

Table 11. Effect of storage on stability of some nonprovitamin A carotenoids

Technology	Product	Bioactive compound	Effect	Ref.
Storage <0°C	Watermelon	(all-*E*)-lycopene	↓	[148]
	Tomato (diced, pulp, puree)	(all-*E*)-lycopene	↓	[120, 149, 150]
	Pizza	(all-*E*)-lycopene	↓	[150, 151]
	Green beans	(all-*E*)-lutein	↓	[152]
	Red grapefruit (juice concentrate)	Total carotenoids	↓	[153]
Storage 0 – 10°C	Tomato	(all-*E*)-lycopene	–	[39]
	Carrot (*Kintoki*)	Total carotenoids	↓	[112]
		(all-*E*)-lycopene	↓	[112]
	Watermelons	(all-*E*)-lycopene	↓	[34]
	Spinach	(all-*E*)-lutein	– (*dark*)	[113]
		(all-*E*)-lutein	↓ (*light*)	[113]
	Endive	(all-*E*)-lutein	↓	[29]
Storage >10°C	Tomato	(all-*E*)-lycopene	↑	[39]
	Tomato (juice, canned juice, paste, soup, sauce, pulp, puree)	(all-*E*)-lycopene	–	[119, 154, 155]
	Tomato (powder)	(all-*E*)-lycopene	↓	[156 – 158]
	Tomato (juice)	(all-*E*)-lutein	↓	[159]
	Red pepper (whole/cut pods, powder)	(all-*E*)-lutein	↓	[135]
	Carrot (spray dried pulp)	(all-*E*)-lutein	↓	[160]
		(9*Z*)-lutein	–	[160]
		(13*Z*)-lutein	↑	[160]

–, No changes; ↑, increase; ↓, decrease.

plays an important role in lycopene stability. Under inert atmosphere (nitrogen) storage, much greater lycopene losses were observed in foam-mat dried tomato powder with a moisture content <1% than in powders with ≈3% moisture content, confirming that the enhancement of oxidative reactions are associated with very low moisture materials [156]. However, in products with higher moisture contents (9 – 23%), an increase of moisture enhances the oxidative lycopene losses [157]. At very low moisture contents, lipid auto-oxidation is enhanced leading to important lycopene losses. In the intermediate moisture range nonenzymatic browning reactions are favoured, which could provide some protection against carotenoid oxidation.

3.4 Summary

It is evident that different processes have different effects on specific carotenoids probably due to: (i) the chemical/stereochemical structure of the carotenoid (*e.g.* carotene, alcohol, epoxide, (*Z*)/(*E*)-isomer), (ii) its integration into a specific food matrices (*e.g.* free or esterified, as crystals or lipid droplets), (iii) the presence of pro-oxidants (Cu^{2+}, Fe^{2+}) and/or antioxidants (ascorbic acid, vitamin E) therein and (iv) its stability upon heating time and temperature, light as well as oxygen. Therefore, it is difficult to assess a general effect of food processing. In conclusion, the effects of thermal processing and storage on stability and bioavailability of carotenoids depend mainly on the severity of the thermal treatments applied. At lower temperatures (60 – 100°C), most carotenoids are stable and isomerization is negligible during blanching, pasteurization, cooking, low temperature drying and frying. Due to the disruption of the matrix of plant tissues and the destruction of the integrity of cell walls and membranes, carotenoid extractability is often increased. At temperatures above 100°C, practised for canning and sterilization, total carotenoid contents are decreased, major (*Z*)-isomerization occurs and bioavailability is improved due to enhanced matrix disruption and oil supplements. The fairly high bioavailability rise at processing temperatures above 100°C may be also due to isomerization rather than matrix disruption alone. In contrast, as an effect of oxygen, carotenoids are instable during drying processes as well as during storage of fresh, dry or frozen products. Further studies about processing and storage effects on carotenoids should focus on specific carotenoids in specific vegetables/fruits with the objective of optimizing industrial processes in order to improve the bioaccessibility and bioavailability of carotenoids (see Section 4).

4 Bioavailability

Bioavailability is defined as the fraction of a dietary component capable of being absorbed and available for use or storage. This is a crucial point in the assessment of the role of provitamins in human health, both to overcome deficiency and to potentially decrease the risk for several chronic diseases.

4.1 Preabsorptive processes and absorption

Studies on absorption of carotenoids started in the early 1960s [161], however the molecular mechanisms involved in their passage through the enterocytes still remain a mat-

ter of debate [162]. Bioaccessibility of carotenoids in vegetables is remarkably low and these compounds are characterized by a slow rate of absorption both in animals and humans because their chemical structure deeply interacts with macromolecules within the plant food matrix [162]. As an example, an *in vitro* digestion model system reported that only 1–3% of the β-carotene in raw carrots is accessible for absorption; and the accessibility of lycopene in canned and fresh tomatoes was <1% [163, 164]. Further studies indicated that more than 70% of the carotenoids remained in the final digesta [165].

4.1.1 Storage factor influencing the release of carotenoids from food matrix

A lot of factors can influence the initial release of carotenoids from the food matrix and their subsequent dissolution in lipidic drop in the stomach and duodenum [166]. Release from the food matrix is the initial and important step in the absorption process of carotenoids. Generally they are present in complexes with proteins as in green leaf vegetables or in semicrystalline structure as in carrots and tomatoes. Then they have to be transferred or dissolved in the lipid phase before they are absorbed. Physically altering food by cooking, blending or finely chopping improves release from the food matrix [132, 164]. Furthermore, the gastric hydrolysis of dietary lipids and proteins increases the release of carotenoids from the food matrix, and begins the process of solubilization of carotenoids into mixed lipid micelles in the gut lumen. The transfer of carotenoids from the predominantly aqueous environment to bulk lipid or micelles requires very close proximity of carotenoids to lipid micelles that starts to happen during the gastric digestion [167]. In this phase, the roles of bile salts and pancreatic secretion are critical for the emulsification, and during solubilization of carotenoids in the mixed micelles. Furthermore, Serrano *et al*. [168], showed a significant inverse correlation between small intestine availability of carotenoids (lutein + β-carotene) and content of klason lignin, nonstarch polysaccharides and resistant protein in green leafy vegetables that should directly affect the intestinal availability of carotenoids acting as a barrier to the action of digestive enzymes and to the release of carotenoids from the food matrix.

Xanthophylls present in fruits, however, seem to be more efficiently released than β-carotene. *In vitro* studies indicated that, in green vegetables, epoxy-xanthophylls and their ester derivatives present in fruits are transferred more easily into the micellar phase [165, 169]. Furthermore, in the case of dietary ester of zeaxanthin, the partial hydrolysis promoted by carboxyl ester lipase during the small intestinal phase of digestion enhances the bioavailability of this carotenoid [170].

4.1.2 Postharvest factors influencing the carotenoid bioavailability

The effect of food processing on carotenoids bioavailability can be illustrated by comparing the blood response after heating a raw food compared with food that has been heat-treated and/or mechanically homogenized to disrupt the food matrix. Stahl and Sies [171] found that boiling tomato juice with 1% corn oil for 1 h before consumption led to a two-fold increase in lycopene plasma concentrations compared to the consumption of tomato juice not further heated. Porrini *et al*. [172] demonstrated that plasma total lycopene levels were higher after the intake of a commercial tomato puree that had undergone a process of heating and homogenization than after raw tomato consumption, thus demonstrating a significant effect of thermal treatment on food matrix and on absorption. On the same way, van het Hof *et al*. [173] observed that both, heating tomato for 1 h at 100°C and homogenization under high pressure, enhanced the lycopene response in both, triglyceride-rich lipoproteins and plasma, significantly. During sterilization of a *Nutri Red* carrot homogenate with a 1% oil supplement at 130°C for 30 min, the isomeric ratio of (all-*E*)- to total (*Z*)-isomers changed from 90:10 to 50:50. Isomeric ratio of the same homogenate, cooked at 100°C for 30 min without oil supplementation, was not altered. For consumption, oil content of all samples was 1%. Compared to the ingestion of an untreated control (blanched and stored at −50°C), a ninefold increase with the lycopene content of the chylomicron fraction was found in the sterilized sample; bioavailability of the cooked samples increased by only 2.5-fold. Although no (5*Z*)-lycopene was generated in the homogenates during any of both thermal treatments, this isomer accounted for about 20% of the total lycopene in chylomicrons [174]. A remarkable enrichment of the relative contents of (5*Z*)-lycopene was also observed after ingestion of tomatoes, tomato juice and purée, respectively [175]. In contrast, lycopene uptake from whole cherry tomatoes, ingested either fresh or cooked at 100°C for 15 min without previous mechanical disruption, was not altered [176].

4.1.3 The composition of the meal on bioavailability

Experimental evidence has been accumulated on the role of dietary fat in the absorption and bioconversion of provitamin A carotenoids to vitamin A [14, 177]. The dietary fat intake plays an important role in the plasma responses to β-carotene supplements [178]. Recently, Brown *et al*. [179] showed that use of fat-free or reduced-fat salad dressings limited the absorption of carotenoids, which are abundant in fresh vegetable salads. In a view of these results, the authors suggested the threshold of 3–5 g fat *per* meal reported by Roodenburg *et al.* [180] and adopted as a guide-

line to promote optimal absorption of β-carotene [181]. In the study by Roodenburg *et al.*, α-carotene and β-carotene were provided in the form of pure supplements dissolved in fat, and not from plant foods. Other investigators used plant sources and found that minimal dietary fat (2.4 g/meal) is sufficient for optimal absorption of provitamin A carotenoids and their bioconversion into vitamin A [9]. The effects of lipid intake on the absorption of carotenoids was confirmed by the observation that the addition of avocado fruit or avocado oil as a lipid source enhances absorption of lycopene and β-carotene and α-carotene, β-carotene and lutein, respectively in humans [182]. Dietary fibre intake is another factor that could regulate carotenoid bioavailability. It is a known fact that fibre decreases the absorption of carotenoids by entrapping them and interacting with bile acids; this leads to an increase of faecal excretion of fats and fat-soluble substances such as carotenoids [183, 184].

The inter-relationship of the different carotenoids present in the food matrix also affects carotenoid absorption. A competitive inhibition, towards the absorption mechanism of a single carotenoid derivative, in fact, may occur at the level of micellar incorporation, intestinal uptake, or lymphatic transport or at one or more of the later steps. It has been proposed that a high-dose intake of carotenoids may antagonize the bioavailability and absorption of other carotenoids. For example, studies on simultaneous ingestion of carotenoids indicate that β-carotene may interfere with absorption of lutein and canthaxanthin, while high doses of simultaneous combination between lycopene and β-carotene decrease bioavailability of both [185, 186]. In contrast, Hoppe *et al*. [187], showed no interaction towards lycopene absorption by β-carotene, β-cryptoxanthin, α-carotene, lutein and zeaxanthin.

4.1.4 Physiological state of the consumer

Parasitism and disease resulting in intestinal dysfunction may have profound effects on carotenoid uptake and bioconversion, but these pathological states have not yet been adequately quantified. For example, in some studies, the lack of observed improvement in vitamin A status in individuals consuming dark green, leafy vegetables may be attributable, at least in part, to concomitant infection with intestinal helminths, *Helicobacter pylori*, or other organisms [188]. Persistent diarrhoea, lipid malabsorption, and deficiencies of vitamin A, protein and zinc also appear to be important factors that impair provitamin A-carotenoid utilization, in addition to their effects on vitamin A metabolism and turnover [166]. Carotenoid-rich fruits and vegetables may indeed provide sufficient vitamin A to meet physiological requirements and even replete body stores under conditions of relatively good health and hygiene. However, debilitating infections and parasitic infestations which are endemic in the tropics and subtropics both compromise carotenoid utilization and increase the individual's requirement for vitamin A. Thus, programs which seek to improve community vitamin A status through food-based interventions will be complemented and strengthened by public health measures which decrease the burden of infection and illness.

Also, age is an other factor that contributes to carotenoid bioavailability [189]. Carroll *et al*. [190] estimated from the analysis FFQ that β-carotene and lycopene are the major dietary carotenoids obtained from a younger and older Irish population. The profile of plasma carotenoid concentrations showed that β-carotene is the major carotenoid in both age groups. Younger groups have higher plasma concentrations of lycopene, β-cryptoxanthin, lutein + zeaxanthin. As described in other European populations these moderate positive associations exist between several plasma carotenoid concentrations and estimated record dietary carotenoids in younger but not in older groups [191].

4.2 Methodology to assess bioavailability

Several confounding factors are present in the literature regarding the assessment of carotenoid bioavailability in humans. Generally the pharmacokinetic studies only provided information on relative bioavailability (relative to reference dose or control) and not on the absolute bioavailability of the carotenoids. Moreover, acute studies need to use large doses of carotenoids to elicit a quantifiable change in blood or urine excretion.

Frequently the approaches used in human studies are short-term, single-dose, pharmacokinetic studies or long-term, multiple-dose supplementation assays. In the latter, the information obtained, relative to nutritional status, depletion and/or saturation processes, could be affected by the typology of the protocol used (*i.e*. on samples collections, ‘acute‘, postprandial metabolism or ‘chronic’) [192]. Furthermore, these studies could be broadly divided into those using large pharmacological doses, which are only partly available due to limitations in the absorption process, and those using more physiological carotenoid doses, either using pure substances, and different matrices, including foods.

Another critical point is the individual response. Based mostly on plasma concentrations observed after carotenoid administration, there is evidence to suggest that there are ‘poor’ and ‘good’ absorbers. This fact is frequently observed in single-dose kinetic studies whereas in long-term studies most of the subjects show significant, though highly variable responses. Thus, this discrimination of subjects based on plasma responses has been criticized since a lack of acute plasma response does not necessarily mean absence of absorption.

Studies of bioavailability of carotenoids, however, are difficult for the endogenous presence in plasma and tissues of carotenoids. In most cases, larger doses than those provided by mixed diets need to be supplied in order to observe variations in plasma. To overcome this problem stable iso-

tope-labelled carotenoids are being increasingly used to assess nutrient bioavailability [193]. In this regard, stable isotope labelling can be performed both intrinsically (in growing foods) and extrinsically (single compounds), allowing the study of carotenoid bioavailability (*i.e.* absorption, transport, distribution, storage, excretion, turn-over, …) at dietary levels and regardless of endogenous presence.

These methods, however, have limitations (*i.e.* the perceived health risk and the costs associated with the necessary methodology). Because of these limitations, many studies have been performed using *in vitro* and animal models. Although animal models may provide relevant information with regard to bioavailability in man, no one animal model completely mimics human absorption and metabolism of carotenoids [194]. Extrapolation of these results and their relevance to humans should, therefore, be considered with caution.

In vitro models based on human physiology have been developed as simple, inexpensive and reproducible tools to study digestive stability, micellization, intestinal transport and metabolism and to predict the bioavailability of different food components. *In vitro* models have been used in studies on vitamin and carotenoid absorption mechanisms and, recently, models of *in vitro* digestion, micellarization and uptake by cell culture (Caco-2 cells) have been used as a model to assess carotenoid bioavailability from foods [195]. This approach is useful for studying preabsorptive processes and thus food related factors that affect bioavailability. Nonetheless, some type of standardization is needed and a wider use of these protocols will determine whether they are valid in predicting absorbability and/or bioavailability in humans.

Finally, an interesting alternative to estimate carotenoid bioavailability could be the evaluation of compartmental modelling that allows us to describe the absorption, redistribution and disposal of nutrients in the body [196, 197].

4.3 Tissue culture experiments for cellular uptake and metabolism

Although the intestinal uptake of carotenoids has been thought to occur by simple diffusion [198], recent studies have reported the existence of protein-mediated transport of carotenoids in enterocytes. Studies in Caco-2 cell monolayers indicate [199 – 201] that carotenoids and cholesterol could share common mechanistic pathways across the intestinal cell. In fact ezetimibe (EZ), an inhibitor of cholesterol transport as well as cholesterol itself inhibited (in a concentration-dependent manner) β-carotene transport, but did not affect retinol transport. This suggests that β-carotene and cholesterol interact during their transport through Caco-2 cells, and, therefore, nonpolar carotenoids and cholesterol share one (or more) common transporter(s). The scavenger receptor type B1 (SR-BI) was postulated to play a role in intestinal cholesterol [202, 203], and β-carotene absorption. In a similar manner, the putative proteins involved in the facilitated diffusion of carotenoids are identified in the Niemann-Pick C1Like 1(NPC1L1) and the adenosine triphosphate (ATP)-binding cassette (ABC) A1 transporter.

A similar *in vitro* system was proposed to study lutein absorption. Lutein was added to Caco-2 cell culture and the absorption of lutein was measured. The rate of transport of lutein micelles (lutein mixed with phospholipids, lysophospholipids, cholesterol, monoolein, oleic acid and taurocholate) was time- and concentration-dependent and was inhibited by coincubation with anti-SR-BI antibody and BLT1 (a leukotriene receptor). Coincubation with β-carotene, but not lycopene, decreased the lutein absorption rate (approx. 20%) significantly. These results suggest that lutein absorption is, at least partly, protein-mediated and that some lutein is taken up through SR-BI [204]. Although a binding protein specific to lycopene has not yet been verified, *in vivo* studies in rats suggested that one may exist. This could explain the preferential uptake of 14C-lycopene in some tissues [205].

Once the carotenoid is inside the enterocyte its fate depends on its structure. If the carotenoid contains an unsubstituted β-ionone ring with a polyene side-chain of at least 11 carbon atoms, it can be cleaved enzymatically to vitamin A. This central cleavage pathway, which requires molecular oxygen, is catalysed by the enzyme carotenoid 15,15′-monooxygenase, and yields two molecules of (all-*E*)-retinal from (all-*E*) β-carotene. This enzyme apparently cleaves (9*Z*) β-carotene also, yielding a 1:1 mixture of (all-*E*) and (9*Z*) retinal, which can be further oxidized to (9*Z*) retinoic acid. The (9*Z*) and (all-*E*) isomers of β-carotene can also be interconverted [206].

The second pathway of β-carotene metabolism is the eccentric cleavage, which occurs at double bonds other than the central 15,15′-double bond of the polyene chain of β-carotene to produce β-apo-carotenals with different chain lengths. However, given that only trace amounts of apocarotenals are detected in *in vivo* treatment [207] and that they can be formed nonenzymatically from β-carotene auto-oxidation [208], the existence of this pathway has been the subject of debate. The two major sites of β-carotene conversion in humans are the intestine and liver. By direct determination of β-carotene oxygenase activity in human small intestine and liver samples, it was estimated that in a human adult the maximum capacity for β-carotene cleavage by the two tissues would be 12 mg β-carotene *per* day [209]; this amount is much higher than the observed average daily intake of 1.5 mg *per* day in the United States or even the higher daily intake of 6 mg β-carotene/day suggested by some authors as being needed to meet the goal of 90% of vitamin A intake [210].

Very little is known about cellular events that regulate or facilitate the incorporation of carotenoids into lymphatic lipoproteins. Still unsolved is how the flow of hydrophobic

carotenoids within the enterocyte is controlled. The poor solubility of carotenoids in aqueous solutions suggests the need for a cytosolic binding protein, but to date no specific binding protein for carotenoids in the intestinal mucosa has been reported. Under normal dietary conditions both the retinyl esters formed from carotenoids in the enterocyte and the intact absorbed carotenoids are incorporated into lymphatic chylomicron [211].

4.4 Human studies

The wide presence of carotenoids in foods have attracted the researchers' attention towards human intervention studies. Up till now, many papers have been published in this area and, considering the wide variety of parameters and factors evaluated, it becomes quite difficult to be exhaustive in the description of the so many aspects of carotenoid bioavailability.

In the EPIC study [212], a typical population groups study, the mean of the sum of the six measured carotenoids (β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin) varied two-fold between regions in men and women (1.35 μmol/L for men in Malmö *vs*. 2.79 μmol/L for men in Ragusa/Naples; 1.61 μmol/L for women in The Netherlands *vs*. 3.52 μmol/L for women in Ragusa/Naples). Women had higher plasma levels of carotenoids than men, except in the case of lycopene. This is in agreement with data reported earlier [74, 213]. Mean carotenoid levels in plasma, in population groups of several regions, showed broader distributions: Italian regions, Athens and UK vegetarians had the highest lycopene and lutein levels while β-carotene and α-carotene were highest among UK vegetarians and β-cryptoxanthin levels were higher in the Spanish regions [212].

Supplementation studies represent another way to test the carotenoids bioavailability in humans; within a multicentre study, serum responses to carotenoid supplementation (lutein, lycopene or α-carotene + β-carotene) were assessed in a randomized, placebo-controlled intervention study [214]. The trial involved 400 apparently healthy men and women (40 men, 40 women/region) from five European regions (France, Northern Ireland, Republic of Ireland, The Netherlands and Spain) and it was conducted using identical time protocols (16 months), capsule preparations and very similar doses (approx. 15 mg carotenoids), allowing relative comparisons between each carotenoid treatment. In addition, the centralization, randomization and quality control of analysis eliminate interlaboratory analytical bias and improve reliability of the results. Carotenoid supplementation was set at dietary achievable levels and then, the supplement of α- and β-carotene supplied an amount equivalent to that contained in 100 g cooked carrots; lutein amount was similar to that present in 200 g cooked spinach and lycopene was equivalent to that provided by 600 g raw tomato or 100 g tomato paste. Data from this study showed that supplementation with α + β-carotene (carotene-rich palm-oil) resulted in a 14- and 5-fold increase in serum levels respectively. Supplementation with lutein (from marigold extracts) elevated serum lutein (about five-fold), zeaxanthin (about double) and ketocarotenoids (not supplied), whereas lycopene supplementation (derived from tomato paste) resulted in a two-fold increase in serum lycopene. Isomer distribution of β-carotene and lycopene in serum remains constant regardless of the isomer composition in the capsules. In Spanish volunteers, additional data [215] showed that serum response to carotenoid supplementation reached a plateau after 4 wks of supplementation whereas no significant side-effects (except carotenodermia) nor changes in biochemical or haematological indices were observed. The presence of a chromatographic peak (tentatively identified as lutein monopalmitate) was only detected in subjects with relatively high serum lutein levels (>1.05 μmol/L). This peak may be indicative of a ceiling effect on saturation of the transport capacity of lutein, which may be re-esterified *in vivo* when it is supplied in excess of normal dietary intake [214, 215].

A lot of human epidemiological studies suggest a protective effect of diets rich in carotenoids, composed mainly of fruit and vegetables, against cancers at various sites. In contrast, intervention studies with higher concentrations of synthetic β-carotene more available than that in fruit and vegetables, have failed to provide the expected protection [216 – 221]. In addition, β-carotene is an important antioxidant in our daily diet which might be significant for health promoting even if its role for disease prevention is still not clear. Concerning lycopene, a correlation between lycopene derived from tomato products supplementation and risk of prostate cancer, was reported by Basu and Imrhan [222] in a recent review of 20 studies, even if future investigations are required to clarify the lycopene role and its action mechanism.

These results suggest that at present there is still insufficient evidence to advocate the consumption of isolated carotenoids for prevention of several chronic diseases [79, 223 – 227]. In fact, data collected with the same methodology, comparable and representing a large number of population are required to quantify the intake of carotenoids and to represent the consumption of the population.

5 Concluding remarks

Carotenoids are a wide variety of molecules present in the human diet so our review is extensive and covers different aspects. The main dietary sources of carotenoids were reviewed. We have tried to summarize positive and negative effects of food processing, storage, cooking on carotenoid bioavailability. In particular, we have evidenced the possibility to improve carotenoids bioavailability in accordance with changes and variations of technology procedures.

We focused our attention on several factors influencing carotenoid accumulation and bioavailability and on the potential health properties and possible biological role of these phytochemicals in human physiology.

The metabolism, absorption and excretion of carotenoids have been studied extensively *in vitro*, in animal models and in humans.

Although a lot of literature data are available for the design and interpretation of intervention studies [228, 229], further investigations are required to understand the absorption and metabolism pathways and the action mechanism of carotenoids in humans. From this point of view, this paper could be a useful updated knowledge for both expert and not expert readers. It also highlights the need for further research with appropriate approaches (*i.e.* dietary intake evaluation, development and update of a carotenoid database for different countries).

The authors are grateful to Editorial Office of British Journal of Nutrition to reuse the Tables 3, 4, 6 and 7.

The authors have declared no conflict of interest.

6 References

[1] West, C.-E., Poortvliet, E.-J., *The Carotenoid Content of Foods With Special Reference to Developing Countries*, Department of Human Nutrition, Wageningen Agricultural University, The Netherlands 1993.

[2] Mangels, A.-R., Holden, J.-M., Beecher, G.-R., Forman, M.-R., Lanza, E., Carotenoid content of fruits and vegetables: An evaluation of analytic data, *J. Am. Diet. Assoc*. 1993, *93*, 284–296.

[3] O'Neill, M.-E., Carroll, Y., Corridan, B., Olmedilla, B., *et al*., A European Carotenoid Database to assess carotenoid intakes and its use in a five-country comparative study, *Br. J. Nutr.* 2001, *85*, 499–507.

[4] U.S. Department of Agriculture, Agricultural Research Service, *USDA National Nutrient Database for Standard Reference*, Release 18, 2005, Nutrient Data Laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp.

[5] Britton, G., Structure and properties of carotenoids in relation to function, *FASEB J.* 1995, *9*, 1551–1558.

[6] di Mascio, P., Kaiser, S., Sies, H., Lycopene as the most efficient biological carotenoid singlet oxygen quencher, *Arch. Biochem. Biophy.* 1989, *274*, 532–538.

[7] Snodderly, D.-M., Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins, *Am. J. Clin. Nutr.* 1995, *62*, 1448S–1461S.

[8] Chrong, E.-W.-I., Wong, T.-Y., Kreis, A.-J., Simpson, J.-A., Guymer, R.-H., Dietary antioxidants and primary prevention of age related macular degeneration: Systemic review and meta-analysis, *Br. Med. J.* 2007, *335*, 755–759.

[9] Ribaya-Mercado, J.-D., Blumberg, J.-B., Lutein and zeaxanthin and their potential roles in disease prevention, *J. Am. Coll. Nutr.* 2004, *23*, 567S–587S.

[10] di Mascio, P., Murphy, M.-E., Sies, H., Antioxidant defense systems: The role of carotenoids, tocopherols, and thiols, *Am. J. Clin. Nutr.* 1991, *53*, 194S–200S.

[11] Bendich, A., Carotenoids and the immune response, *J. Nutr.* 1989, *119*, 112–115.

[12] Mathews-Roth, M.-M., Plasma concentrations of carotenoids after large doses of beta-carotene, *Am. J. Clin. Nutr.* 1990, *52*, 500–501.

[13] Nishino, H., Cancer prevention by carotenoids, *Mutat. Res*. 1998, *402*, 159–163.

[14] Khachik, F., Sprangler, C.-J., Smith, J.-C., Canfield, L.-M., *et al*., Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum, *Anal. Chem*. 1997, *69*, 1873–1881.

[15] Holden, J.-M., Eldridge, A.-L., Beecher, G.-R., Buzzard, M., *et al*., Carotenoid content of U.S. foods: An update of the database, *J. Food Comp. Anal*. 1999, *12*, 169–196.

[16] Hart, D.-J., Scott, K.-J., Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruit commonly consumed in the UK, *Food Chem*. 1995, *54*, 101–111.

[17] Leth, T., Jakobsen, J., Andersen, N.-L., The intake of carotenoids in Denmark, *Eur. J. Lipid Sci. Technol*. 2000, *102*, 128–132.

[18] Murkovic, M., Gams, K., Draxl, S., Pfannhauser, W., Development of an Austrian carotenoid database, *J. Food Comp. Anal*. 2000, *13*, 435–440.

[19] Sass-Kiss, A., Kiss, J., Milotay, P., Kerek, M.-M., Toth-Markus, M., Differences in anthocyanin and carotenoid content of fruits and vegetables, *Food Res. Intern*. 2005, *38*, 1023–1029.

[20] Lugasi, A., Biro, L., Hovarie, J., Sagi, K.-V., *et al*., Lycopene content of foods and lycopene intake in two groups of the Hungarian population, *Nutr. Res*. 2003, *23*, 1035–1044.

[21] Dragovic-Uzelac, V., Levaj, B., Mrkic, V., Bursac, D., Boras, M., The content of polyphenols and carotenoids in three apricot cultivars depending on stage of maturity and geographical region, *Food Chem*. 2007, *102*, 966–975.

[22] Lu, Q.-Y., Arteaga, J.-R., Zhang, Q., Huerta, S., *et al*., Inhibition of prostate cancer cell growth by an avocado extract: Role of lipid-soluble bioactive substances, *J. Nutr. Biochem*. 2005, *16*, 23–30.

[23] Setiawan, B., Sulaeman, A., Giraud, D.-W., Driskell, J.-A., Carotenoid content of selected Indonesian fruits, *J. Food Comp. Anal*. 2001, *14*, 169–176.

[24] Wall, M.-M., Ascorbic acid, vitamin A, and mineral composition of banana (*Musa* sp.) and papaya (*Carica papaya*) cultivars grown in Hawaii, *J. Food Comp. Anal*. 2006, *19*, 434–445.

[25] Larsen, E., Christensen, L.-P., Simple saponification method for the quantitative determination of carotenoids in green vegetables, *J. Agric. Food Chem*. 2005, *53*, 6598–6602.

[26] Niizu, P.-Y., Rodriguez-Amaya, D.-B., New data on the carotenoid composition of raw salad vegetables, *J. Food Comp. Anal*. 2005, *18*, 739–749.

[27] Kidmose, U., Yang, R.-Y., Thilsted, S.-H., Christensen, L.-P., Brandt, K., Content of carotenoids in commonly consumed Asian vegetables and stability and extractability during frying, *J. Food Comp. Anal*. 2006, *19*, 562–571.

[28] Kimura, M., Rodriguez-Amaya, D.-B., Carotenoid composition of hydroponic leafy vegetables, *J. Agric. Food Chem*. 2003, *51*, 2603–2607.

[29] de Azevedo-Meleiro, C.-H., Rodriguez-Amaya, D.-B., Carotenoids of endive and New Zealand spinach as affected by maturity, season and minimal processing, *J. Food Comp. Anal*. 2005, *18*, 845 – 855.

[30] Su, Q., Rowley, K.-G., Itsiopoulos, C., O'Dea, K., Identification and quantitation of major carotenoids in selected components of the Mediterranean diet: Green leafy vegetables, figs and olive oil, *Eur. J. Clin. Nutr*. 2002, *56*, 1149 – 1154.

[31] Kopsell, D.-A., Kopsell, D.-E., Curran-Celentano, J., Carotenoid pigments in kale are influenced by nitrogen concentration and form, *J. Sci. Food Agric*. 2007, *87*, 900 – 907.

[32] Frenich, A.-G., Torres, M.-E.-H., Vega, A.-B., Vidal, J.-L.-M., Bolanos, P.-P., Determination of ascorbic acid and carotenoids in food commodities by liquid chromatography with mass spectrometry detection, *J. Agric. Food Chem*. 2005, *53*, 7371 – 7376.

[33] Melendez-Martinez, A.-J., Vicario, I.-M., Heredia, F.-J., Provitamin A carotenoids and ascorbic acid contents of the different types of orange juices marketed in Spain, *Food Chem*. 2007, *101*, 177 – 184.

[34] Gil, M.-I., Aguayo, E., Kader, A.-A., Quality changes and nutrient retention in fresh-cut versus whole fruit during storage, *J. Agric. Food Chem*. 2006, *54*, 4284 – 4296.

[35] Guil-Guerrero, J.-L., Martínez-Guirado, C., Rebolloso-Fuentes, M.-D.-M., Carrique-Pérez, A., Nutrient composition and antioxidant activity of 10 pepper (*Capsicum annuun*) varieties, *Eur. Food Res. Technol*. 2006, *224*, 1 – 9.

[36] Kornsteiner, M., Wagner, K.-H., Elmadfa, I., Tocopherols and total phenolics in 10 different nut types, *Food Chem*. 2006, *98*, 381 – 387.

[37] Bellomo, M.-G., Fallico, B., Anthocyanins, chlorophylls and xanthophylls in pistachio nuts (*Pistacia vera*) of different geographic origin, *J. Food Comp. Anal*. 2007, *20*, 352 – 359.

[38] Bergquist, S.-Å.-M., Gertsson, U.-E., Olsson, M.-E., Influence of growth stage and postharvest storage on ascorbic acid and carotenoid content and visual quality of baby spinach (*Spinacia oleracea* L.), *J. Sci. Food Agric*. 2006, *86*, 346 – 355.

[39] Toor, R.-K., Savage, G.-P., Changes in major antioxidant components of tomatoes during post-harvest storage, *Food Chem*. 2006, *99*, 724 – 727.

[40] Lenucci, M.-S., Cadinu, D., Taurino, M., Piro, G., Dalessandro, G., Antioxidant composition in cherry and high-pigment tomato cultivars, *J. Agric. Food Chem*. 2006, *54*, 2606 – 2613.

[41] Raffo, A., Malfa, G.-L., Fogliano, V., Maiani, G., Quaglia, G., Seasonal variations in antioxidant components of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1), *J. Food Comp. Anal*. 2006, *19*, 11 – 19.

[42] Sanchez-Moreno, C., Plaza, L., de Ancos, B., Cano, M.-P., Nutritional characterisation of commercial traditional pasteurised tomato juices: Carotenoids, vitamin C and radical-scavenging capacity, *Food Chem*. 2006, *98*, 749 – 756.

[43] Ishida, B.-K., Chapman, M.-H., A comparison of carotenoid content and total antioxidant activity in Catsup from several commercial sources in the United States, *J. Agric. Food Chem*. 2004, *52*, 8017 – 8020.

[44] Hulshof, P.-J. M., van Roekel-Jansen, T., van de Bovenkamp, P., West, C.-E., Variation in retinol and carotenoid content of milk and milk products in The Netherlands, *J. Food Comp. Anal*. 2006, *19*, 67 – 75.

[45] Perkins-Veazie, P., Collins, J.-K., Davis, A.-R., Roberts, W., Carotenoid content of 50 watermelon cultivars, *J. Agric. Food Chem*. 2006, *54*, 2593 – 2597.

[46] Toor, R.-K., Savage, G.-P., Lister, C.-E., Seasonal variations in the antioxidant composition of greenhouse grown tomatoes, *J. Food Comp. Anal*. 2006, *19*, 1 – 10.

[47] Caldwell, C.-R., Britz, S. J., Effect of supplemental ultraviolet radiation on the carotenoid and chlorophyll composition of green house-grown leaf lettuce (*Lactuca sativa* L.) cultivars, *J. Food Comp. Anal*. 2006, *19*, 637 – 644.

[48] de Pee, S., West, C. E., Dietary carotenoids and their role in combating vitamin A deficiency: A review of the literature, *Eur. J. Clin. Nutr*. 1996, *50*, S38 – S53.

[49] Welch, R.-M., Agronomic problems related to provitamin A carotenoid-rich plants, *Eur. J. Clin. Nutr*. 1997, *51*, S34 – S38.

[50] Solomons, N.-W., Bulux, J., Identification and production of local carotene-rich foods to combat vitamin A malnutrition, *Eur. J. Clin. Nutr*. 1997, *51*, S39 – S45.

[51] Kidmose, U., Knuthsen, P., Edelenbos, M., Justesen, U., Hegelund, E., Carotenoids and flavonoids in organically grown spinach (*Spinacia oleracea* L) genotypes after deep frozen storage, *J. Sci. Food Agric*. 2001, *81*, 918 – 923.

[52] Xu, C.-J., Fraser, P.-D., Wang, W.-J., Bramley, P.-M., Differences in the carotenoid content of ordinary citrus and lycopene-accumulating mutants, *J. Agric. Food Chem*. 2006, *54*, 5474 – 5481.

[53] Rajyalakshmi, P., Venkatalaxmi, K., Venkatalakshmamma, K., Jyothsna, Y., Devi, K.-B., Suneetha, V., Total carotenoid and beta-carotene contents of forest green leafy vegetables consumed by tribals of south India, *Plant Foods Hum. Nutr*. 2001, *56*, 225 – 238.

[54] Furtado, J., Siles, X., Campos, H., Carotenoid concentrations in vegetables and fruits common to the Costa Rican diet, *Int. J. Food Sci. Nutr*. 2004, *55*, 101 – 113.

[55] Lako, J., Trenerry, V.-C., Wahlqvist, M., Wattanapenpaiboon, N., *et al*., Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods, *Food Chem*. 2007, *101*, 1727 – 1741.

[56] Scott, K.-J., Observations on some of the problems associated with the analysis of carotenoids in foods by HPLC, *Food Chem*. 1992, *45*, 357 – 364.

[57] Shi, J., Le Maguer, M., Lycopene in tomatoes: Chemical and physical properties affected by food processing, *Crit. Rev. Biotechnol*. 2000, *20*, 293 – 334.

[58] Rodríguez-Bernaldo de Quirós, A., Costa, H.-S., Analysis of carotenoids in vegetable and plasma samples: A review, *J. Food Comp. Anal*. 2006, *19*, 97 – 111.

[59] Oliver, J., Palou, A., Chromatographic determination of carotenoids in foods, *J. Chromatogr. A* 2000, *881*, 543 – 555.

[60] Calvo, M.-M., Lutein: A valuable ingredient of fruit and vegetables, *Crit. Rev. Food Sci. Nutr*. 2005, *45*, 671 – 696.

[61] Seo, J.-S., Burri, B.-J., Quan, Z., Neidlinger, T.-R., Extraction and chromatography of carotenoids from pumpkin, *J. Chromatogr. A* 2005, *1073*, 371 – 375.

[62] Howe, J.-A., Tanumihardjo, S.-A.-J., Evaluation of analytical methods for carotenoid extraction from biofortified maize (*Zea mays* sp.), *Agric. Food Chem*. 2006, *54*, 7992 – 7997.

[63] Panfili, G., Fratianni, A., Irano, M., Improved normal-phase high-performance liquid chromatography procedure for the determination of carotenoids in cereals, *J. Agric. Food Chem*. 2004, *52*, 6373 – 6377.

[64] Burns, J., Fraser, P.-D., Bramley, P.-M., Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables, *Phytochemistry* 2003, *62*, 939 – 947.

[65] Sander, L.-C., Wise, S.-A., in: Smith, R.-M. (Ed.), *Retention and Selectivity Studies in HPLC*, Elsevier, Amsterdam 1994, pp. 337.

[66] Sander, L.-C., Sharpless, K.-E., Pursch, M., C30 Stationary phases for the analysis of food by liquid chromatography, *J. Chromatogr. A* 2000, *880*, 189 – 202.

[67] Schieber, A., Carle, R., Occurrence of carotenoid cis-isomers in food: Technological, analytical, and nutritional implications, *Trends Food Sci. Technol*. 2005, *9*, 416 – 422.

[68] AOAC, The Association Of Analytical Communities, Official Method 941.15, *Carotene in Fresh Plant Materials and Silages*, 2007; AOAC Official Method 970.64, *Carotenes and Xanthophylls in Dried Plant Materials and Mixed Feeds*, 2007.

[69] CEN, European Committee for Standardization, *Foodstuffs – Determination of vitamin A by high performance liquid chromatography – Part 2: Measurement of* β*-carotene*, EN 12823-2.: 1-13. CEN, Brussels 1999.

[70] Brevik, A., Andersen, L.-F., Karlsen, A., Trygg, K.-U., *et al*., Six carotenoids in plasma used to assess recommended intake of fruits and vegetables in a controlled feeding study, *Eur. J. Clin. Nutr*. 2004, *58*, 1166 – 1173.

[71] Riboli, E., Pèquignot, G., Repetto, F., Axerio, M., *et al*., A comparative study of smoking, drinking and dietary habits in population samples in France, Italy, Spain and Switzerland. I. Study design and dietary habits, *Rev. Epidemiol. Santé Publique* 1988, *36*, 151 – 165.

[72] Ascherio, A., Stampfer, M., Colditz, G.-A., Rimm, E.-B., *et al*., Correlations of vitamin A and E intakes with plasma concentrations of carotenoids and tocopherols among American men and women, *J. Nutr*. 1992, *122*, 1792 – 1801.

[73] Scott, K.-J., Thurnham, D.-I., Hart, D., The correlation between the intake of lutein, lycopene and β-carotene from vegetables and fruits and blood plasma concentrations in a group of women aged 50 – 66 years in the UK, *Br. J. Nutr*. 1996, *75*, 409 – 418.

[74] Olmedilla, B., Granado, F., Blanco, I., Rojas-Hidalgo, E., Seasonal and sex-related variations in six serum carotenoids, retinol, and alpha-tocopherol, *Am. J. Clin. Nutr*. 1994, *60*, 106 – 110.

[75] Olmedilla, B., Granado, F., Southon, S., Wright, A.-J., *et al*., Serum concentrations of carotenoids, vitamins A, E, and C, in control subjects from five European countries, *Br. J. Nutr*. 2001, *85*, 227 – 238.

[76] Granado, F., Olmedilla, B., Blanco, I., Rojas-Hidalgo, E., Major fruit and vegetable contributors to the main serum carotenoids in the Spanish diet, *Eur J. Clin. Nutr*. 1996, *50*, 246 – 250.

[77] Granado, F., Blázquez, S., Olmedilla, B., Changes in carotenoid intake from fruit and vegetables in the Spanish population over the period 1964–2004, *Public Health Nutr*. 2007, *19*, 1 – 6.

[78] Elia, M., Stratton, R.-J., Geographical inequalities in nutrients status and risk of malnutrition among English people aged 65 year and older, *Nutrition* 2005, *21*, 1100 – 1106.

[79] Howard, A. N., Williams, N. R., Palmer, C. R., Cambou, J. P., *et al*., Do hydroxyl-carotenoids prevent coronary heart disease? A comparison between Belfast and Toulouse, *Int. J. Vit. Nutr. Res.* 1996, *66*, 113 – 118.

[80] Byrd-Bredbenner, C., Lagiou, P., Trichopoulou, A., A comparison of household food availability in 11 countries, *J. Hum. Nutr. Dietet*. 2000, *13*, 197 – 204.

[81] Elmadfa, I., Weichselbaum, E. (Eds.), *European Nutrition and Health Report: Forum of Nutrition, 2004: Energy and Nutrient Intake in the European Union*, Karger, Basel 2005, Vol. 58, pp. 19 – 46.

[82] Naska, A., Fouskakis, D., Oikonomou, E., Almeida, M.-D., *et al*., Dietary patterns and their socio-demographic determinants in 10 European countries: Data from the DAFNE databank, *Eur. J. Clin. Nutr*. 2006, *60*, 181 – 190.

[83] FAOSTAT, FAO Statistical Database, *Food Balance Sheets* 2005.

[84] Granado, F., Olmedilla, B., Blanco, I., Gil-Martínez, E., Rojas-Hidalgo, E., Variability in the intercomparison of food carotenoid content data: A user's point of view, *Crit. Rev. Food Sci. Nutr*. 1997, *37*, 621 – 633.

[85] Deharveng, G., Charrondere, U.-K., Slimani, N., Southgate, D.-A.-T., Riboli, E., Comparison of nutrients in the food composition tables available in the nine European countries participating in EPIC, *Eur. J. Clin. Nutr*. 1999, *53*, 60 – 79.

[86] van den Berg, H., Faulks, R., Granado, F., Hirschberg, J., *et al*., The potential for the improvement of carotenoid levels in foods and the likely systemic effects, *J. Sci. Food Agric*. 2000, *80*, 880 – 913.

[87] van Dokkum, W., de Vos, R.-H. (Eds.), *EURONUT Report No. 9*, TNO-CIVO, Zeist, Netherlands 1987.

[88] Granado, F., Olmedilla, B., Blanco, I., Nutritional and clinical relevance of lutein in human health, *Br. J. Nutr*. 2003, *90*, 487 – 502.

[89] Heinonen, M.-I., Ollilainen, V., Linkola, E.-K., Varo, P.-T., Koivistoinen, P.-E., Carotenoids in finnish foods: Vegetables, fruits, and berries, *J. Agric. Food Chem*. 1989, *37*, 655 – 659.

[90] Tee, E.-S., Lim, C.-L., Carotenoid composition and content of Malaysian vegetables and fruits by the AOAC and HPLC methods, *Food Chem*. 1991, *41*, 309 – 339.

[91] Granado, F., Olmedilla, B., Blanco, I., Rojas-Hidalgo, E., Carotenoid composition in raw and cooked spanish vegetables, *J. Am. Food Chem*. 1992, *40*, 2135 – 2140.

[92] Poorvliet, E.-J., West, C.-E., *The Carotenoid Content of Foods With Special Reference to Developing Countries, Vitamin A, Field Support Project (VITAL)*, International Science and Technology Institute, Inc., Arlington, VA 1993.

[93] Olmedilla, B., Granado, F., Blanco, I., Gil-Martìnez, E., Rojas-Hidalgo, E., *Contenido de Carotenoides en Verduras y Frutas de Mayor Consumo en España*, Instituto Nacional de la Salud (INSALUD), Secretarìa General, Madrid (España) 1996.

[94] Sommerburg, O., Keunen, J.-E., Bird, A.-C., van Kuijk, F.-J., Fruits and vegetables that are sources for lutein and zeaxanthin: The macular pigment in human eyes, *Br. J. Ophthalmol*. 1998, *82*, 907 – 910.

[95] Pattison, D.-J., Symmons, D.-P., Lunt, M., Welch, A., *et al*., Dietary beta-cryptoxanthin and inflammatory polyarthritis: Results from a population-based prospective study, *Am. J. Clin. Nutr*. 2005, *82*, 451 – 455.

[96] Männistö, S., Smith-Warner, S.-A., Spiegelman, D., Albanes, D., *et al*., Dietary carotenoids and risk of lung cancer in a pooled analysis of seven cohort studies, *Cancer Epidemiol. Biomarkers Prev*. 2004, *13*, 40 – 48.

[97] Gascón-Vila, P., Ribas, L., García-Closas, R., Farrán Codina, A., Serra-Majem, L., Dietary sources of vitamin A, C, E and beta-carotene in a adult Mediterranean population, *Gac. Sanit*. 1999, *13*, 22 – 29.

[98] Garcia-Closas, R., Gonzalez, C.-A., Agudo, A., Riboli, E., Intake of specific carotenoids and flavonoids and the risk of gastric cancer in Spain, *Cancer Causes Control*. 1999, *10*, 71 – 75.

[99] García-Closas, R., Berenguer, A., José Tormo, M., José Sánchez, M., *et al*., Dietary sources of vitamin C, vitamin E and specific carotenoids in Spain, *Br. J. Nutr*. 2004, *91*, 1005 – 1011.

[100] Lucarini, M., Lanzi, S., D'Evoli, L., Aguzzi, A., Lombardi-Boccia, G., Intake of vitamin A and carotenoids from the Italian population-results of an Italian total diet study, *Int. J. Vitam. Nutr. Res*. 2006, *76*, 103 – 109.

[101] van den Berg, H., Carotenoid interactions, *Nutr. Rev.* 1999, *57*, 1 – 10.

[102] Granado, F., Olmedilla, B., Herrero, C., Pérez-Sacristán, B., Blázquez, S., *Bioaccesibility of phytochemicals from vegetables: Effect of minimal processing (modified atmospheres)*, Innovations in Traditional Foods, INTRAFOOD 2005, Congress Proceedings, Vol. II, Valencia, Spain 2005, pp. 1147 – 1150.

[103] van Dokkum, W., de Vos, R.-H., Schrijve, J., Retinol, total carotenoids, β-carotene and tocopherols in total diets of male adolescents in The Netherlands, *J. Agric. Food Chem*. 1190, *38*, 221 – 226.

[104] Jarvinen, R., Carotenoids, retinoids, tocopherols and tocotrienals in the diet: The Finnish Mobile Clinic Health Examination Survey, *Int. J. Vit. Nutr. Res*. 1995, *65*, 24 – 30.

[105] Hedrén, E., Diaz, V., Svanberg, U., Estimation of carotenoid accessibility from carrots determined by an *in vitro* digestion method, *Eur. J. Clin. Nutr*. 2002, *56*, 425 – 430.

[106] Ribeiro, H.-S., Guerrero, J.-M.-M., Briviba, K., Rechkemmer, G., *et al*., Cellular uptake of carotenoid-loaded oil-in-water emulsions in colon carcinoma cells *in vitro*, *J. Agric. Food Chem*. 2006, *54*, 9366 – 9369.

[107] Hornero-Méndez, D., Mínguez-Mosquera, M.-M., Bioaccessibility of carotenes from carrots: Effect of cooking and addition of oil, *Innvat. Food Sci. Emerg. Technol*. 2007, *8*, 407 – 412.

[108] Brown, M.-J., Ferruzzi, M.-G., Nguyen, M.-L., Cooper, D.-A., *et al*., Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection, *Am. J. Clin. Nutr*. 2004, *80*, 396 – 403.

[109] Huo, T., Ferruzzi, M.-G., Schwartz, S.-J., Failla, M.-L., Impact of fatty acyl composition and quantity of tryglycerides on bioaccessibility of dietary carotenoids, *J. Agric. Food Chem*. 2007, *55*, 8950 – 8957.

[110] Lindley, M.-G., The impact of food processing on antioxidants in vegetable oils, fruits and vegetables, *Trends Food Sci. Technol*. 1998, *9*, 336 – 341.

[111] Serafini, M., Bugianesi, R., Salucci, M., Azzini, E., *et al*., Effect of acute ingestion of fresh and stored lettuce (*Lactuca sativa*) on plasma total antioxidant capacity and antioxidant levels in human subjects, *Br. J. Nutr*. 2002, *88*, 615 – 623.

[112] Mayer-Miebach, E., Spieß, W.-E.-L., Influence of cold storage and blanching on the carotenoid content of Kintoki carrots, *J. Food Eng*. 2003, *56*, 211 – 213.

[113] Kopas-Lane, L.-M., Warthesen, J.-J., Carotenoid stability in raw spinach and carrots during cold storage, *J. Food Sci*. 1995, *60*, 773 – 776.

[114] Aman, R., Biehl, J., Carle, R., Conrad, J., *et al*., Application of HPLC coupled with DAD, APcI-MS and NMR to the analysis of lutein and zeaxanthin stereoisomer in thermally processed vegetables, *Food Chem*. 2005, *92*, 753 – 763.

[115] Choe, E., Lee, J., Park, K., Lee, S., Effects of heat pretreatment on lipid and pigments of freeze-dried spinach, *J. Food Sci*. 2001, *66*, 1074 – 1079.

[116] Sanchez-Moreno, C., Plaza, L., de Arcos, B., Cano, M. P., Impact of high-pressure and traditional thermal processing of tomato purée on carotenoid, vitamin C and antioxidant activity, *J. Sci. Food Agric*. 2006, *86*, 171 – 179.

[117] Lee, H.-S., Coates, G.-A., Effect of thermal pasteurisation on Valencia orange juice colour and pigments, *Lebensm. Wiss. Technol*. 2003, *36*, 153 – 156.

[118] Torregrosa, F., Cortés, C., Esteve, M.-J., Frígola, A., Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids, *J. Agric. Food Chem*. 2005, *53*, 9519 – 9525.

[119] Agarwal, A., Shen, H., Agarwal, S., Rao, A.-V., Lycopene content of tomato products: Its stability, bioavailability and *in vivo* antioxidant properties, *J. Med. Food* 2001, *4*, 9 – 15.

[120] Sharma, S.-K., Le Maguer, M., Kinetics of lycopene degradation in tomato pulp solids under different processing and storage conditions, *Food Res. Intern*. 1996, *29*, 309 – 315.

[121] Mayer-Miebach, E., Behsnilian, D., Regier, M., Schuchmann, H.-P., Thermal processing of carrots: Lycopene stability and isomerisation with regard to antioxidant potential, *Food Res. Intern*. 2005, *38*, 1103 – 1108.

[122] Khachik, F., Goli, M.-B., Beecher, G.-R., Holden, J., *et al*., Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables, *J. Agric. Food Chem*. 1992, *40*, 390 – 398.

[123] Anese, M., Falcone, P., Fogliano, V., Nicoli, M.-C., Massini, R., Effect of equivalent thermal treatments on the colour and the antioxidant activity of tomato purée, *J. Food Sci*. 2002, *67*, 3442 – 3446.

[124] Updike, A.-A., Schwartz, S.-J., Thermal processing of vegetables increases cis-isomers of lutein and zeaxanthin, *J. Agric. Food Chem*. 2003, *51*, 184 – 6190.

[125] Scott, C.-E., Eldridge, A.-L., Comparison of carotenoid content in fresh, frozen and canned corn, *J. Food Comp. Anal*. 2005, *18*, 551 – 559.

[126] Mayer-Miebach, E., Behsnilian, D., Gräf, V., Neff, E., *et al*., *Optimising the carotenoid content of processed products based on a lycopene rich carrot*, 4th European Congress in Chemical Engineering, September 21 – 25, Granada, Spain 2003.

[127] Regier, M., Mayer-Miebach, E., Behsnilian, D., Neff, E., Schuchmann, H.-P., Influences of drying and storage of lycopene-rich carrots on the carotenoid content, *Dry. Technol*. 2005, *23*, 989 – 998.

[128] Kerkhofs, N., Lister, C., Savage, G., Change in colour and antioxidant content of tomato cultivars following forced-air drying, *Plant. Foods Hum. Nutr*. 2005, *60*, 117 – 121.

[129] Zanoni, B., Peri, C., Nani, R., Lavelli, V., Oxidative heat damage of tomato halves as affected by drying, *Food Res. Intern*. 1998, *31*, 395 – 401.

[130] Toor, R.-K., Savage, G.-P., Effect of semi-drying on the antioxidant components of tomatoes, *Food Chem*. 2006, *94*, 90 – 97.

[131] Goula, A.-M., Adamopoulos, K.-G., Stability of lycopene during spray drying of tomato pulp, *Food Sci. Technol*. 2005, *38*, 479–487.

[132] Lavelli, V., Hippeli, S., Peri, C., Elstner, E.-F., Evaluation of radical scavenging activity of fresh and air-dried tomatoes by three model reactions, *J. Agric. Food Chem*. 1999, *47*, 3826–3831.

[133] Idda, P., Behsnilian, D., Schuchmann, H.-P., Carotinoidstabilität bei der thermischen Verarbeitung von zeaxanthinreichen transgenen Kartoffeln, *Chem. Ing. Tech.* 2005, *77*, 1193–1194.

[134] Minguez-Mosquera, M.-I., Pérez-Gálvez, A., Garrido-Fernandez, J., Carotenoid content of the varieties Jaranda and Jariza (*Capsicum annuum* L.) and response during the industrial slow drying and grinding steps in paprika processing, *J. Agric. Food Chem*. 2000, *48*, 2972–2976.

[135] Kim, S., Park, J., Hwang, I.-K., Composition of main carotenoids in Korean red pepper (*Capsicum annuum* L.) and changes of pigment stability during the drying and storage process, *J. Food Sci*. 2004, *69*, C39–C44.

[136] Perez-Galvez, A., Hornero-Mendez, D., Minguez-Mosquera, M.-I., Changes in the carotenoid metabolism of capsicum fruit during application of modified slow drying process for paprika production, *J. Agric. Food Chem*. 2004, *52*, 518–522.

[137] Topuz, A., Ozdemir, F., Influences of γ-irradiation and storage on the carotenoids of sun-dried and dehydrated paprika, *J. Agric. Food Chem*. 2003, *51*, 4972–4977.

[138] Konschuh, M., McAllister, T., Dalpé, S., Lewis, T., *et al*., *Assessment of Carotenoid Content of Yellow-Fleshed Potato Varieties Grown in Alberta to Determine Potential Nutritional Benefits, Final Report Project: 2004-008*, Alberta Agricultural, Food and Rural Development, Alberta 2005, pp. 1–19.

[139] Sulaeman, A., Keeler, L., Giraud, D. W., Taylor, S. L., *et al*., Carotenoid content and physicochemical and sensory characteristics of carrot chips deep-fried in different oils at several temperatures, *J. Food Sci*. 2001, *66*, 1257–1264.

[140] Zhang, D., Hamauzu, Y., Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking, *Food Chem*. 2004, *88*, 503–509.

[141] Chen, B.-H., Chen, Y.-Y., Stability of chlorophylls and carotenoids in sweet potato leaves during microwave cooking, *J. Agric. Food Chem*. 1993, *41*, 1315–1320.

[142] de Ancos, B., Cano, M.-P., Hernandez, A., Monreal, M., Effects of microwave heating on pigment composition and colour of fruit purées, *J. Sci. Food Agric*. 1999, *79*, 663–670.

[143] Abushita, A.-A., Daood, H.-G., Biacs, P.-A., Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors, *J. Agric. Food Chem*. 2000, *48*, 2075–2081.

[144] Takeoka, G.-R., Dao, L., Flessa, S., Gillespie, D.-M., *et al*., Processing effects on lycopene content and antioxidant activity of tomatoes, *J. Agric. Food Chem*. 2001, *49*, 3713–3717.

[145] Torres Gama, J.-J., de Sylos, C.-M., Effect of thermal pasteurization and concentration on carotenoid composition of Brazilian Valencia orange juice, *Food Chem*. 2007, *100*, 1686–1690.

[146] Dewanto, V., Wu, X., Adom, K.-K., Liu, R.-H., Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity, *J. Agric. Food Chem*. 2002, *50*, 3010–3014.

[147] Behsnilian, D., Mayer-Miebach, E., Idda, P., Schuchmann, H.-P., *Thermal stability of zeaxanthin in a genetically modified potato*, 4th International Congress on Pigments in Food, Hohenheim University, Stuttgart, Germany, October 9–12, 2006.

[148] Fish, W.-W., Davis, A.-R., The effects of frozen storage conditions on lycopene stability in watermelon tissue, *J. Agric. Food Chem*. 2003, *51*, 3582–3585.

[149] Lisiewska, Z., Kmiecik, W., Effect of storage period and temperature on the chemical composition and organoleptic quality of frozen tomato cubes, *Food Chem*. 2000, *70*, 167–173.

[150] Cremona, F., Sandei, L., Taddei, C., Leoni, C., Evaluation, over time, of freezing effects in lycopene content and colour of frozen tomato products, *Ind. Cons*. 2004, *79*, 379–395.

[151] Taddei, C., Sandei, L., Cremona, F., Leoni C., Evaluation over the effects, over time, of freezing on lycopene content and on the colour of frozen pizza surfaces, *Ind. Cons*. 2005, *80*, 235–256.

[152] Oruña-Concha, M.-J., González-Castro, M.-J., López-Hernández, J., Simal-Lozano, J., Effects of freezing on the pigment content in green beans and padrón peppers, *Eur. Food Res. Technol*. 1997, *205*, 148–153.

[153] Lee, H.-S., Coates, G.-A., Characterisation of colour fade during frozen storage of red grapefruit juice concentrates, *J. Agric. Food Chem*. 2002, *50*, 3988–3991.

[154] Lavelli, V., Giovanelli, G., Evaluation of heat and oxidative damage during storage of processed tomato products. II. Study of oxidative damage indices, *J. Sci. Food Agric*. 2003, *83*, 966–971.

[155] Nguyen, M.-L., Francis, D., Schwartz, S., Thermal isomerisation susceptibility of carotenoids in different tomato varieties, *J. Sci. Food Agric*. 2001, *81*, 910–917.

[156] Lovric, T., Sablek, S., Boskovic, M., Cis-trans isomerisation of lycopene and colour stability of foam-mat dried tomato powder during, *J. Sci. Food Agric*. 1970, *21*, 641–647.

[157] Giovanelli, G., Paradiso, A., Stability of dried and intermediate moisture tomato pulp during storage, *J. Agric. Food Chem*. 2002, *50*, 7277–7281.

[158] Anguelova, T., Warthesen, J., Lycopene stability in tomato powders, *J. Food Sci*. 2000, *65*, 67–70.

[159] Lin, C.-H., Chen, B.-H., Stability of carotenoids in tomato juice during storage, *Food Chem*. 2005, *90*, 837–846.

[160] Chen, B.-H., Tang, Y.-C., Processing and stability of carotenoid powder from carrot pulp waste, *J. Agric. Food Chem*. 1998, *46*, 2312–2318.

[161] Glover, J., The conversion of β-carotene into vitamin A, in: Harris, R.-S., Ingle, D.-J. (Eds.), *Vitamins and Hormones*, Vol. 18, Academic Press, New York and London 1960, pp. 371–386.

[162] van het Hof, K.-H., West, C.-E., Weststrate, J.-A., Hautvast, J.-G.-A.-J., Dietary factors that affect the bioavailability of carotenoids, *J. Nutr*. 2000, *10*, 503–506.

[163] Stahl, W., van den Berg, H., Arthur, J., Bast, A., *et al*., Bioavailability and metabolism, *Mol. Aspects Med*. 2002, *23*, 39–100.

[164] Hedrén, E., Diaz, V., Svanberg, U., Estimation of carotenoid accessibility from carrots determined by an *in vitro* digestion method, *Eur. J. Clin. Nutr*. 2002, *56*, 425–430.

[165] Granado-Lorencio, F., Olmedilla-Alonso, B., Herrero-Barbudo, C., Blanco-Navarro, I., *et al*., *In vitro* bioaccessibility of carotenoids and tocopherols from fruits and vegetables, *Food Chem*. 2007, *102*, 641 – 648.

[166] Erdman, J., Bierer, T.-L., Gugger, E.-T., Absorption and transport of carotenoids, *Ann. N.Y. Acad. Sci*. 1993, *691*, 76 – 85.

[167] Tyssandier, V., Reboul, E., Dumas, J.-F., Bouteloup-Demange, C., *et al*., Processing of vegetable-borne carotenoids in the human stomach and duodenum, *Am. J. Physiol. Gastrointest. Liver Physiol*. 2003, *284*, G913 – G923.

[168] Serrano, J., Goñi, I., Saura-Calixto, F., Determination of beta-carotene and lutein available from green leafy vegetables by an *in vitro* digestion and colonic fermentation method, *J. Agric. Food Chem*. 2005, *20*, 2936 – 2940.

[169] Granado, F., Olmedilla, B., Herrero, C., Pérez-Sacristán, B., Blázquez, *In vitro model to assess bioaccesibility of carotenoids from fruits*, Innovations in Traditional Foods INTRAFOOD 2005, Congress Proceedings, Vol. II, 2005, pp. 1150 – 1154.

[170] Chitchumroonchokchai, C.-H., Failla, M., Hydrolysis of zeaxanthin esters by carboxyl ester lipase during digestion facilitates micellarization and uptake of the xanthophylls by Caco-2 human cells, *J. Nutr*. 2006, *136*, 588 – 594.

[171] Stahl, W., Sies, H., Uptake of lycopene and its geometrical isomers is greater from heat-precessed than from unprocessed tomato juice in humans, *J. Nutr*. 1992, *122*, 2161 – 2166.

[172] Porrini, M., Riso, P., Testolin, G., Absorption of lycopene from single or daily portions of raw and processed tomato, *Br. J. Nutr*. 1998, *80*, 353 – 361.

[173] van het Hof, K.-H., de Boer, B.-C., Tijburg, L.-B., Lucius, B.-R., *et al*., Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption, *J. Nutr*. 2000, *130*, 1189 – 1196.

[174] Mayer-Miebach, E., Behsnilian, D., Schuchmann, H.-P., Bub, A., Isomerisation of lycopene due to thermal treatment of carrot homogenates: Increased bioavailability of total lycopene and generation of 5-cis-lycopene in the human intestine, in: Senate Commission on Food Safety SKLM (Ed.), *Thermal Processing of Food – Potential Health Benefits and Risks, Forschungsberichte (DFG)*, Wiley-VCH, Weinheim 2007.

[175] Fröhlich, K., Kaufmann, K., Bitsch, R., Böhm, V., Effects of ingestion of tomatoes, tomato juice and tomato purée on contents of lycopene isomers, tocopherols and ascorbic acid in human plasma as well as on lycopene isomer pattern, *Br. J. Nutr*. 2006, *95*, 734 – 741.

[176] Bugianesi, R., Salucci, M., Leonardi, C., Ferracane, R.-M., *et al*., Effect of domestic cooking of naringenin, chlorogenic acid, lycopene and β-carotene in cherry tomatoes, *Eur. J. Nutr*. 2004, *43*, 360 – 366.

[177] Prince, M.-R., Frisoli, J.-K., Beta-carotene accumulation in serum and skin, *Am. J. Clin. Nutr*. 1993, *57*, 175 – 181.

[178] Dimitrov, N.-V., Meyer, C., Ullrey, D.-E., Chenoweth, W., *et al*., Bioavailability of beta-carotene in humans, *Am. J. Clin. Nutr*. 1988, *48*, 298 – 304.

[179] Brown, M.-J., Ferruzzi, M.-G., Nguyen, M.-L., Cooper, D.-A., *et al*., Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection, *Am. J. Clin. Nutr*. 2004, *80*, 396 – 403.

[180] Roodenburg, A.-J., Leenen, R., van het Hof, K.-H., Weststrate, J.-A., Tijburg, L.-B., Amount of fat in the diet affects bioavailability of lutein esters but not of alpha-carotene, beta-carotene, and vitamin E in humans, *Am. J. Clin. Nutr*. 2000, *71*, 1029 – 1030.

[181] Nestel, P., Nalubola, R., *As little as one teaspoon of dietary fat in a meal enhances the absorption of* β*-carotene*, ILSI (The International Life Sciences Institute), Washington, DC 2003.

[182] Unlu, N.-Z., Bohn, T., Clinton, S.-K., Schwartz, S.-J., Carotenoid absorption from salad and salsa by humans is enhanced by the addition of avocado or avocado oil, *J. Nutr*. 2005, *135*, 431 – 436.

[183] Rock, C.-L., Swendseid, M.-E., Plasma beta-carotene response in humans after meals supplemented with dietary pectin, *Am. J. Clin. Nutr*. 1992, *55*, 96 – 99.

[184] Hoffmann, J., Linseisen, J., Riedl, J., Wolfram, G., Dietary fiber reduces the antioxidative effect of a carotenoid and alpha-tocopherol mixture on LDL oxidation ex vivo in humans, *Eur. J. Nutr*. 1999, *38*, 278 – 285.

[185] Kostic, D., White, W.-S., Olson, J.-A., Intestinal absorption, serum clearance and interaction between lutein and beta-carotene when administered to human adults in separate or combined oral doses, *Am. J. Clin. Nutr*. 1995, *62*, 604 – 610.

[186] van den Berg, H., Van Vliet, T., Effect of simultaneous, single oral doses of beta-carotene with lutein or lycopene on the beta-carotene and retinyl ester responses in the triacylglycerol-rich lipoprotein fraction of men, *Am. J. Clin. Nutr*. 1998, *68*, 82 – 89.

[187] Hoppe, P.-P., Krämer, K., van den Berg, H., Steenge, G., van Vliet, T., Synthetic and tomato-based lycopene have identical bioavailability in humans, *Eur. J. Nutr*. 2003, *42*, 272 – 278.

[188] dePees, S., West, C.-E., Muhilal, Karyadi, D., Hautvast, J.-G., Lack of improvement in vitamin A status with increased consmption of dark green leafy vegetables, *Lancet* 1995, *346*, 75 – 81.

[189] Maiani, G., Mobarhan, S., Ceccanti, M., Ranaldi, L., *et al*., Beta-carotene serum response in young and elderly females, *Eur. J. Clin. Nutr*. 1989, *43*, 749 – 761.

[190] Carroll, Y.-L., Corridan, B.-M., Morrissey, P.-A., Carotenoids in young and elderly healthy humans: Dietary intakes, biochemical status and diet-plasma relationships, *Eur. J. Clin. Nutr*. 1999, *53*, 644 – 653.

[191] Jansen, M.-C., Van Kappel, A.-L., Ocke, M.-C., Van't Veer, P. *et al*., Plasma carotenoid levels in Dutch men and women, and the relation with vegetable and fruit consumption, *Eur. J. Clin. Nutr*. 2004, *58*, 1386 – 1395.

[192] Granado-Lorencio, F., Olmedilla-Alonso, B., Bioavailability of vitamins, in: Vaquero, M. P., García-Arias, T., Carbajal, A., Sánchez-Muñiz (Eds.), *Bioavailability of Micronutrients and Minor Dietary Compounds. Metabolic and Technological Aspects. Recent Research Developments in Nutrition*, Research Singpost, Kerala, India 2003, 37/661(2), pp. 19 – 30.

[193] Tang, G., Qin, J., Dolnikowski, G.-G., Russell, R.-M., Grusak, M.-A., Spinach or carrots can supply significant amounts of vitamin A as assessed by feeding with intrinsically deuterated vegetables, *Am. J. Clin. Nutr*. 2005, *82*, 821 – 828.

[194] Lee, C.-M., Boileau, A.-C., Boileau, T.-W., Williams, A.-W., *et al*., Review of animal models in carotenoid research, *J. Nutr*. 1999, *129*, 2271 – 2277.

[195] Failla, M.-L., Chitchumroonchokchai, C., Ishida, B.-K., Swanson, K.-S., *et al*., *In vitro* micellarization and intestinal cell uptake of cis isomers of lycopene exceed those of all-trans lycopene, *J. Nutr*. 2008, *138*, 482 – 486.

[196] Novotny, J.-A., Zech, L.-A., Furr, H.-C., Dueker, S.-R., Clifford, A. J., Mathematical modeling in nutrition: Constructing a physiologic compartmental model of the dynamics of beta-carotene metabolism, *Adv. Food Nutr. Res*. 1996, *40*, 25 – 54.

[197] Green, M.-H., Green, J.-B., The use of model-based compartmental analysis to study vitamin A metabolism in a non-steady state, *Adv. Exp. Med. Biol*. 2003, *537*, 159 – 172.

[198] El-Gorab, M.-I., Underwood, B.-A., Loerch, J.-D., The roles of bile salts in the uptake of β-carotene and retinol by rat everted gut sacs, *Biochim. Biophys. Acta* 1975, *401*, 265 – 277.

[199] During, A., Dawson, H.-D., Harrison, E.-H., Carotenoid transport is decreased and expression of the lipid transporters SR-BI, NPC1L1, and ABCA1 is downregulated in Caco-2 cells treated with ezetimibe, *J. Nutr*. 2005, *135*, 2305 – 2312.

[200] During, A., Harrison, E.-H., Intestinal absorption and metabolism of carotenoids: Insights from cellculture, *Arch. Biochem. Biophys*. 2004, *430*, 77 – 88.

[201] van Bennekum, A., Werder, M., Thuahnai, S.-T., Han, C.-H., *et al*., Class B scavenger receptor-mediated intestinal absorption of dietary beta-carotene and cholesterol, *Biochemistry* 2005, *44*, 4517 – 4525.

[202] Hauser, H., Dyer, J.-H., Nandy, A., Vega, M.-A., *et al*., Identification of a receptor mediating absorption of dietary cholesterol in the intestine, *Biochemistry* 1998, *37*, 843 – 850.

[203] Drobnik, W., Lindenthal, B., Lieser, B., Ritter, M., *et al*., ATP-binding cassette transporter A1 (ABCA1) affects total body sterol metabolism, *Gastroenterology* 2001, *120*, 1203 – 1211.

[204] Reboul, E., Abou, L., Mikail, C., Ghiringhelli, O., *et al*., Lutein transport by Caco-2 TC-7 cells occurs partly by a facilitated process involving the scavenger receptor class B type I (SR-BI), *Biochem. J*. 2005, *387*, 455 – 461.

[205] Zaripheh, S., Erdman, J.-W., The biodistribution of a single oral dose of [14C]-lycopene in rats prefed either a control or lycopene-enriched diet, *J. Nutr*. 2005, *135*, 2212 – 2218.

[206] Furr, H.-C., Clark, R.-M., Intestinal absorption and tissue distribution of carotenoids, *J. Nutr. Biochem*. 1997, *8*, 364 – 377.

[207] Barua, A.-B., Olson, J.-A., Beta-carotene is converted primarily to retinoids in rats *in vivo*, *J. Nutr*. 2000, *130*, 1996 – 2001.

[208] Handelman, G.-J., van Kuijk, F.-J., Chatterjee, A., Krinsky, N.-I., Characterization of products formed during the autoxidation of beta-carotene, *Free Radic. Biol. Med*. 1991, *10*, 427 – 437.

[209] During, A., Smith, M.-K., Piper, J.-B., Smith, J.-C., Beta-Carotene 15,15′-Dioxygenase activity in human tissues and cells: Evidence of an iron dependency, *J. Nutr. Biochem*. 2001, *12*, 640 – 647.

[210] Lachance, P.-A., Future vitamin and antioxidant RDAs for health promotion, *Prev. Med*. 1996, *25*, 46 – 47.

[211] Harrison, E.-H., Mechanisms of digestion and absorption of dietary vitamin A, *Ann. Rev. Nutr*. 2005, *25*, 87 – 103.

[212] Al-Delaimy, W.-K., van Kappel, A.-L., Ferrari, P., Slimani, N., *et al*., Plasma levels of six carotenoids in nine European countries: Report from the European Prospective Investigation into Cancer and Nutrition (EPIC), *Public Health Nutr*. 2004, *7*, 713 – 722.

[213] Rautalahti, M., Albanes, D., Haukka, J., Roos, E., *et al*., Seasonal variation of serum concentrations of beta-carotene and alpha-tocopherol, *Am. J. Clin. Nutr*. 1993, *57*, 551 – 556.

[214] Olmedilla, B., Granado, F., Southon, S., Wright, A.-J.-A., *et al*., European multicenter, placebo-controlled intervention trial with a-tocopherol, carotene rich palm-oil, lutein or lycopene at dietary achievable levels, *Clin. Sci*. 2002, *102*, 447 – 456.

[215] Granado, F., Olmedilla, B., Gil-Martínez, E., Blanco, I., Lutein ester in serum after lutein supplementation in humans, *Br. J. Nutr*. 1998, *80*, 445 – 449.

[216] ATBC Cancer Prevention Study Group, The alpha-tocopherol, beta-carotene lung cancer prevention study: Design, methods, participant characteristics, and compliance, *Ann. Epidemiol*. 1994, *4*, 1 – 10.

[217] Steinmetz, K.-A., Potter, J.-D., Vegetables, fruit and cancer prevention: A review, *J. Am. Diet. Assoc.* 1996, *96*, 1027 – 1039.

[218] IARC, The International Agency for Research on Cancer, *Handbooks of Cancer Prevention. Vol. 2: Carotenoids*, Lyon 1998.

[219] Klipstein-Grobusch, K., Geleijnse, J.-M., den Breeijen, J.-H., Boeing, H., *et al*., Dietary antioxidants and risk of myocardial infarction in the elderly: The Roterdam study, *Am. J. Clin. Nutr*. 1999, *69*, 261 – 266.

[220] Giovannucci, E., Ascherio, A., Rimm, E.-B., Stampfer, M.-J., *et al*., Intake of carotenoids and retinol in relation to risk of prostate cancer, *J. Natl. Cancer Inst*. 1995, *87*, 1767 – 1776.

[221] Chang, S., Erdman, J.-W., Clinton, S.-K., Vadiveloo, M., *et al*., Relationship between plasma carotenoids and prostate cancer, *Nutr. Cancer* 2005, *53*, 127 – 134.

[222] Basu, A., Imrhan, V., Tomatoes versus lycopene in oxidative stress and carcinogenesis: Conclusions from clinical trials, *Eur. J. Clin. Nutr*. 2007, *61*, 295 – 303.

[223] Krinsky, N.-I., Deneke, S.-M., Interaction of oxygen and oxy-radicals with carotenoids, *J. Natl. Cancer Inst*. 1982, *69*, 205 – 210.

[224] Ames, B.-N., Shigenaga, M.-K., Hagen, T.-M., Oxidants, antioxidants, and degenerative diseases of aging, *Proc. Natl. Acad. Sci*. 1993, *90*, 7915 – 7922.

[225] Rock, C.-L., Saxe, G.-A., Ruffin, M.-T., August, D.-A., Schottenfeld, D., Carotenoids, vitamin A, and estrogen receptor status in breast cancer, *Nutr. Cancer* 1996, *25*, 281 – 296.

[226] Khachik, F., Beecher, G.-R., Smith, J.-C., Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer, *J. Cell. Biochem*. 1995, *22*, 236 – 246.

[227] Morris, D.-L., Kritchevsky, S.-B., Davis, C.-E., Serum carotenoids and coronary heart disease. The Lipid Research Clinics Coronary Primary Prevention Trial and Follow-up Study, *JAMA* 1994, *272*, 1434 – 1441.

[228] Pratt, S., Dietary prevention of age-related macular degeneration, *J. Am. Optom. Assoc*. 1999, *70*, 39 – 47.

[229] Bone, R.-A., Landrum, J.-T., Mayne, S.-T., Gomez, C.-M. *et al*., Macular pigment in donor eyes with and without AMD: A case-control study, *Invest. Ophthalmol. Vis. Sci*. 2001, *42*, 235 – 240.

