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Intestinal absorption of lycopene from different foods

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Abstract

Lycopene is the main carotenoid in tomatoes. With its eleven linearly arranged conjugated double bonds it shows high antioxidant activity. Recent epidemiological studies implicate lycopene in the prevention of cardiovascular diseases and cancer. Human intervention studies (6 w or 4 w intervention period) with 5 mg/d or 12.5 mg/d of lycopene comprised in tomatoes, tomato juice, tomato purée and tomato oleoresin capsules showed a good intestinal absorption of lycopene. These results indicate an improved availability of lycopene comprised in heated or processed food due to the dissociation of the protein-carotenoid complexes. Additionally, lipids in the diet increase the absorption of carotenoids. In another intervention study (4 w) with 5 mg/d of lycopene comprised in rosehip purée, surprisingly an increase in the content of lycopene in plasma was not observed during supplementation. However, rubixanthin, a carotenoid special for rosehip, was absorbed during the intervention period. Heating or processing of food seems to be important to increase the intestinal absorption of lycopene. Thus, a new human pilot study was started with heated rosehip purée. An increase in the concentration of lycopene in plasma was not observed either. The content of non-starch polysaccharides in rosehips might be one limiting factor for the absorption of lycopene.

Introduction

Lycopene, an acyclic carotenoid with eleven linearly arranged conjugated double bonds, is found only in few foods. Tomatoes and tomato products are the main foodstuffs contributing to the dietary intake of lycopene. Additionally, guavas, watermelons, papayas, and pink grapefruits as well as rosehip products and sea-buckthorn products contain lycopene. Lycopene lacks the β-ionone ring structure and therefore does not have any provitamin A activity. Recent epidemiological studies implicate lycopene in the prevention of cardiovascular diseases and cancer (Klipstein-Grobusch et al., 2000; Giovannucci et al., 2002).

Recently, the intestinal intake of lycopene has been investigated, using daily dosages of 12 to 75 mg (Brown et al., 1989; Micozzi et al., 1992; Gärtner et al., 1997; Agarwal and Rao, 1998; Paetau et al., 1998; Porrini et al., 1998; Müller et al., 1999; Allen et al., 2003; Hoppe et al., 2003). In the time period, only few other studies used lower daily amounts of lycopene, supplementing with 5 mg/d (Böhm and Bitsch, 1999), 5.7 mg/d (Porrini et al., 2005), 7 mg/d (Porrini and Riso, 2000) and 8 mg/d (Riso et al., 2004), being more related to the physiological lycopene uptake. Hininger et al. (2001) published 4.8 ± 0.4 mg/d as daily lycopene intake from food for 175 volunteers from five European study centers. Most results (**Table 1**) except two studies with tomato juice (Brown et al., 1989; Micozzi et al., 1992) indicate an improved availability of lycopene comprised in heated or processed food. This effect is caused by the dissociation of the protein-carotenoid complexes. Additionally, lipids in the diet increase the absorption of carotenoids.

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Table 1. Human studies on the intestinal absorption of lycopene from tomatoes, tomato products and lycopene capsules

Dosage	Matrix	Result	Reference
12 mg, single dose	• Tomato juice (180 mL)	No change of plasma lycopene compared to baseline	Brown et al., 1989
12 mg/d, 6 w	• Tomato juice (180 mL/d)	No change of plasma lycopene compared to baseline	Micozzi et al., 1992
12 mg/d, 4 w 17 mg/d, 4 w 21 mg/d, 4 w	• Tomato soup (1 cup/d) • Tomato juice (8 oz/d) • Tomato sauce (0.5 cup/d)	Lycopene in plasma ↑	Allen et al., 2003
15 mg/d, 4 w	• Capsules (synthetic lycop.) • Capsules (natural lycop.)	Lycopene in serum ↑	Hoppe et al., 2003
16,5 mg/d, single dose + 7 d	• Tomato purée (60 g/d) • Tomatoes (300 g/d)	Lycopene more available from purée	Porrini et al., 1998
23 mg, single dose	• Tomato paste (40 g) • Tomatoes (400 g)	Lycopene in chylomicrons after tomato paste ↑	Gärtner et al., 1997
39 - 75 mg/d, 1 w	• Spaghetti sauce (126 g/d) • Tomato juice (540 mL/d) • Lycopene capsules	Lycopene in serum ↑	Agarwal and Rao, 1998
40 mg/d, 2 w	• Tomato juice (330 mL/d)	Lycopene in plasma ↑	Müller et al., 1999
70 - 75 mg/d, 4 w	• Tomato juice (476 g/d) • Beadlets • Capsules	Lycopene in plasma ↑	Paetau et al., 1998
5 mg/d, 6 w	• Tomatoes (80 - 230 g/d) • Tomato juice (59 g/d) • Capsules	Lycopene more available from tomato juice and capsules	Böhm and Bitsch, 1999
5.7 mg/d, 26 d	• Tomato oleoresin drink (250 mL/d)	Lycopene in plasma ↑	Porrini et al., 2005
7 mg/d, 2 w	• Tomato purée (25 g/d)	Lycopene in plasma ↑	Porrini and Riso, 2000
8 mg/d, 3 w	• Tomato sauce (60 g/d) • Tomato paste (15 g/d)	Lycopene in plasma ↑	Riso et al., 2004

Lycopene from tomatoes and tomato-based foods exists predominantly in *(all-E)*-configuration, the thermodynamically most stable form (Porrini et al., 1998). In contrast, various *(Z)*-isomers account for over 50% of blood lycopene and for over 75% of tissue lycopene (Clinton et al., 1996; Ferruzzi et al., 2001). The processes that influence isomer patterns and the mechanisms of interconversion are still an essentially unexplored area of research. Isomerisa-

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tion of lycopene may have significant consequences since the large three-dimensional differences between these geometric isomers may influence their pharmacological properties (Holloway et al., 2000). Recent investigations using the TEAC (Trolox equivalent antioxidant capacity) assay showed significantly different antioxidant activity for lycopene isomers depending on the geometrical structure (Böhm et al., 2002).

A recent human intervention study investigated the interrelationships among the intake of different commonly consumed tomato products (tomatoes, tomato juice, tomato purée) and plasma lycopene isomer profiles. All volunteers were supplied with a daily dosage of 12.5 mg lycopene for four weeks after a two weeks diet low in lycopene (Fröhlich et al., 2006). Total-lycopene in plasma decreased in all groups during two-weeks of depletion during which the participants consumed a diet low in lycopene. Ingestion of tomato juice as well as that of tomato purée resulted in an increase of lycopene in plasma within one week (**Fig. 1**). Surprisingly, also the uptake of tomatoes led within two weeks of intervention to increased contents of total-lycopene in plasma, mainly caused by cutting the tomatoes to small pieces before eating them.

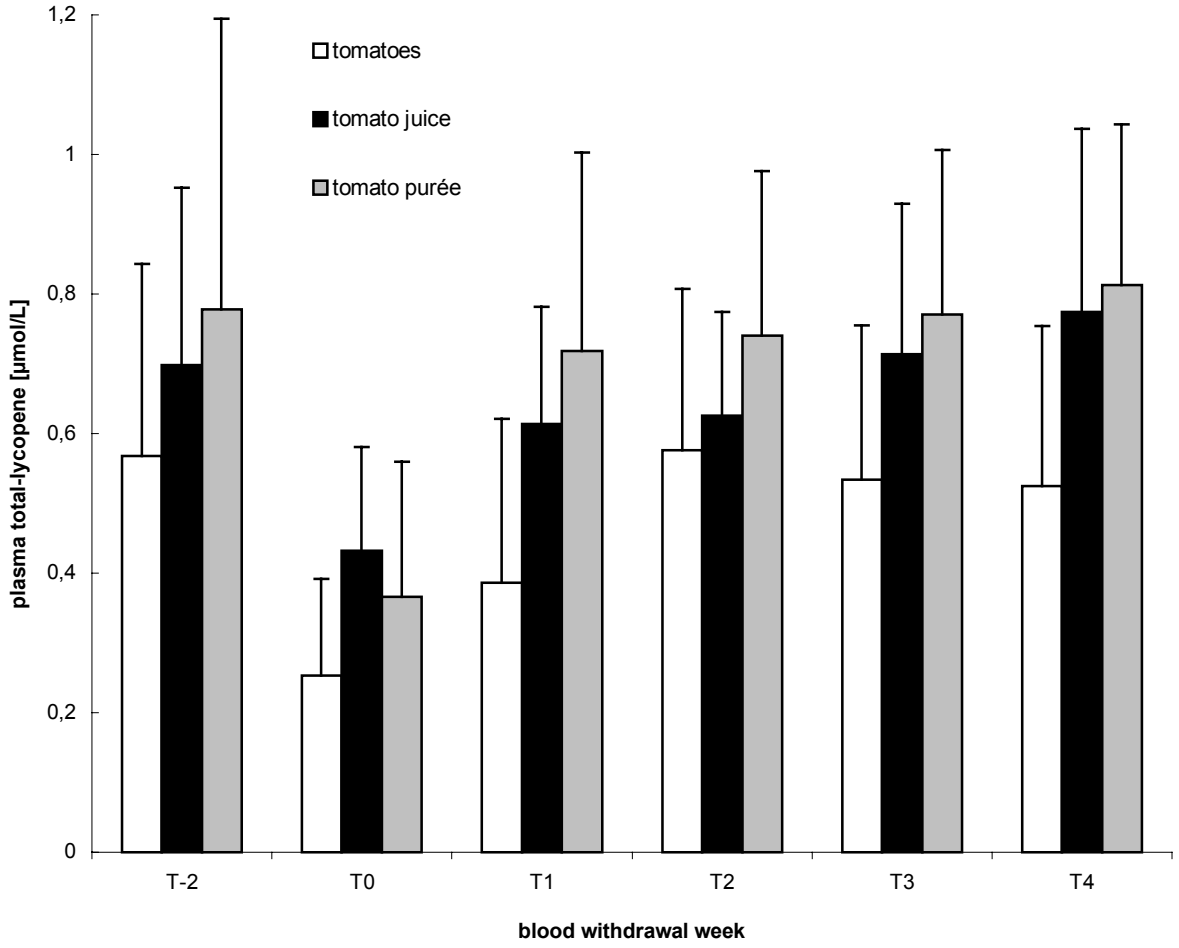

Fig. 1. Plasma total-lycopene concentration (mean values and standard deviations) over time in subjects consuming daily portions of tomatoes, tomato juice or tomato purée for four weeks after a two weeks depletion period.

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The two major isomers in plasma of all volunteers were (*all-E*)- and (*5Z*)-lycopene. The ratios of the sum of all evaluated lycopene (*Z*)-isomers:(*all-E*)-lycopene were used for assessment of isomer changes in plasma. Plasma isomer concentration showed an approximately 60:40 ratio of (*Z*):(*all-E*) at the start of the study. After a 2-week depletion period, the ratios changed. A decrease in the (*all-E*)-configuration to approximately 30% of total lycopene and a compensatory increase of the (*Z*)-isomers to 70% was observed. After 4 weeks of intervention with tomato juice (63% (*Z*); 37% (*all-E*)) and tomato purée (61% (*Z*); 39% (*all-E*)) isomer ratios returned to those observed at the start of the study. After 4 weeks of intervention with raw tomatoes the (*Z*):(*all-E*) ratio was 50:50 (Fröhlich et al. 2006). All these results from recent investigations with tomatoes and tomato products were the background to start human intervention studies with rosehip purée, another source of lycopene. In study 1 a rosehip purée was used with unknown processing conditions. The two rosehip purées used in study 2 were heated purées.

Subjects and methods

Subjects and study design

Five healthy subjects (three women and two men) ranging from 20 to 24 y with a BMI between 19 and 26 kg/m^2 participated in study 1. Four healthy subjects (two women and two men) ranging from 25-26 y (BMI: 19-26 kg/m^2) participated in study 2. All participants were non-smokers and did not take carotenoid supplements or vitamin A supplements. Informed written consent was obtained from each participant and the protocol was approved by the Ethical Committee of the Friedrich Schiller University Jena at the Medical Faculty. Subjects were asked to follow precise instructions regarding their diet to limit carotenoid intake without interfering with their own eating habits. All subjects avoided food rich in lycopene such as tomatoes and tomato products, water melons, yellow and red peppers, pink grapefruits, papayas, guavas, rosehip products and sea-buckthorn products for a 2-week depletion period and the following 4 weeks of intervention. After the depletion period, they ingested 5 mg lycopene/d, comprised in two portions of 38 g (study 1) or 52/55 g (study 2) rosehip purée (one portion during the morning and the second one during the afternoon). To improve the absorption the subjects were asked to ingest each portion of rosehip purée together with 2.5 g sunflower oil. The volunteers were allowed to mix the purée and the oil with other food (e.g. yoghurt or milk). The lycopene content of the rosehip purées was analysed before the start of the study in order to calculate the equivalent amounts for the participants. The purée used in study 1 had a content of total-lycopene of 6.6 ± 1.6 mg/100 g. The total-lycopene content of the other purées (study 2) was 4.8 ± 4.6/4.5 ± 2.2 mg/100 g.

Collection of blood samples

Fasting blood samples (10 mL) were withdrawn from the participants in EDTA tubes before the study (T-2), after 2 weeks of depletion (T0) and thereafter weekly (study 1) while supplemented (T1, T2, T3, T4) or after two (T2) and four weeks (T4) of intervention (study 2). The blood samples were centrifuged at 3500 rpm for 10 min at 4 °C. All plasma samples were stored at –80 °C until analysis. Blood samples and plasma samples were always handled under subdued light.

Analysis of carotenoids

Carotenoids were extracted according to Bieri et al. (1985), slightly modified. An equal volume of ethanolic echinenone (CaroteNature, Lupsingen, Switzerland) solution (internal stan-

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dard) was added to 500 µL of plasma. The sample was mixed using a vortex for 30 sec prior to addition of 250 µL hexane with 0.1% BHT. The mixture was shaken for 1 min and centrifuged at 14 000 rpm for 2 min. The plasma extraction procedure was performed thrice on each sample to ensure total removal of carotenoids. The combined hexane layers were evaporated to dryness using a gentle stream of nitrogen at 30 ± 1 °C. The residue was dissolved in 250 µL of methanol/methyl tert-butyl ether (1+1, v/v), vortexed and centrifuged at 14 000 rpm for 4 min. The supernatant was analysed on a C_{30} (250 x 4.6 mm, 5 µm) column (Trentec, Gerlingen, Germany), preceded by a C_{18} ProntoSil 120-5-C18 H (10 x 4.0 mm, 5 µm) column (Bischoff, Leonberg, Germany) at 17 ± 1 °C with diode array detection at 470 nm (slightly modified to Böhm, 2001). As mobile phase (1.3 mL min^{-1}) the following gradient procedure was used consisting of methanol (solvent A) and methyl tert-butyl ether (solvent B): 1) Initial conditions 90% solvent A and 10% solvent B, 2) a 35-min linear gradient to 45% solvent B, 3) a 10-min linear gradient to 55% solvent B, 4) 45% solvent A and 55% solvent B for 15 min, 5) a 10-min linear gradient to 10% solvent B. All experiments were carried out under subdued light to prevent photo-degradation and isomerisation. Recovery of the internal standard was 85 ± 14% (study 1, n = 105) or 87 ± 8% (study 2, n = 48). (*all-E*)-Lycopene was identified using reference material, which was a kind gift of BASF, Ludwigshafen, Germany. (*all-E*)-Lycopene stock solution in cyclohexane/toluene (4+1, v/v) of 83 µg/mL was prepared and diluted daily 1:100 using a mixture of methanol and methyl tert-butyl ether (1+1, v/v) to get the working solution. The concentration of the stock solution was checked periodically by using its extinction coefficient (E(1%, 1 cm): 3450 (n-hexane, 472 nm), (Craft et al., 1988)). The lycopene (*Z*)-isomers were quantified by using the (*all-E*)-lycopene calibration. Different spectroscopic techniques were used to identify the main lycopene (*Z*)-isomers (Fröhlich et al., 2005). (*all-E*)-Rubixanthin (Apin Chemicals, Abingdon, UK) solutions were prepared similar to the procedure described for lycopene. The concentration of the stock solution was checked periodically by using its extinction coefficient (E(1%, 1 cm): 2750 (petroleum ether, 460 nm), (Britton et al. 2004)).

Rosehip purées were analysed on their carotenoid contents as recently described for tomato products elsewhere (Seybold et al., 2004).

Results

Study 1

After the two weeks diet with low lycopene intake, the content of total-lycopene in plasma significantly ($p < 0.05$) decreased from 0.54 ± 0.19 µmol/L to 0.26 ± 0.15 µmol/L. Surprisingly, an increase of lycopene in plasma was not observed during the supplementation with rosehip purée. Total-lycopene decreased significantly to 0.20 ± 0.07 µmol/L after the four weeks intervention period (**Fig. 2**).

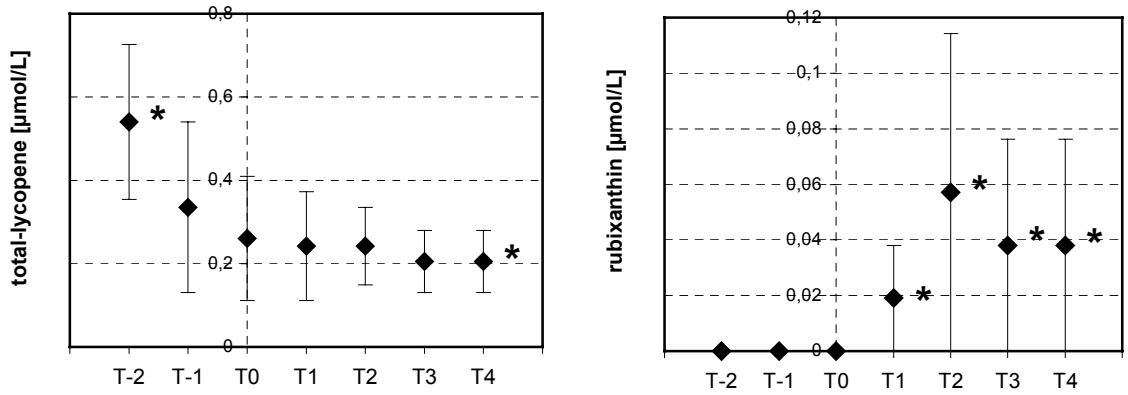
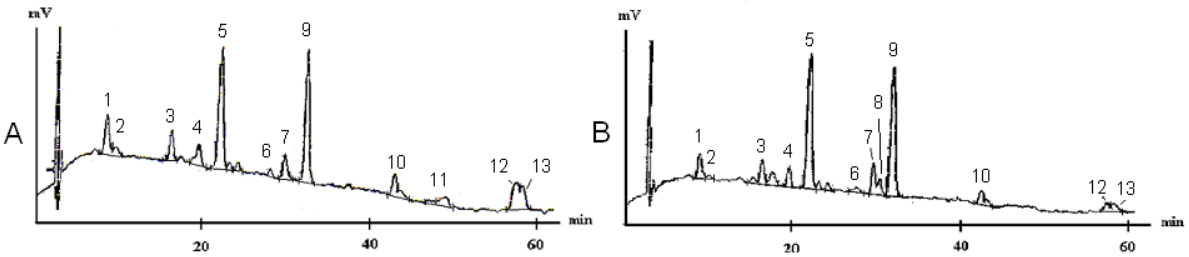


Fig. 2. Plasma contents of total-lycopene and (*all-E*)-rubixanthin (mean values and standard deviations) over time in subjects consuming daily portions of rosehip purée for four weeks after a two weeks depletion period, * significantly different to T0 ($p < 0.05$)

This study showed that lycopene from the consumed rosehip purée was not bioavailable. Contrary to lycopene, a good bioavailability of rubixanthin was reflected in the significant increase of its levels in plasma after the intervention with rosehip purée (Fig. 2). Rubixanthin, a specific carotenoid of the fruits from wild roses and not present in other foodstuffs, was not detected in human plasma at the beginning of the intervention. Its contents in plasma significantly increased to 0.02 ± 0.02 µmol/L after one week and to 0.08 ± 0.04 µmol/L after four weeks of supplementation with rosehip purée. Regarding other carotenoids (lutein, zeaxanthin, canthaxanthin, β-cryptoxanthin, α-carotene, β-carotene), no significant differences were observed in contents in plasma during the entire study period (data not shown). **Fig. 3** shows HPLC chromatograms of human plasma extracts before (A) and after (B) supplementation with rosehip purée.



1 = (all-E)-lutein, 2 = (all-E)-zeaxanthin, 3 = (all-E)-canthaxanthin, 4 = (all-E)-ß-cryptoxanthin, 5 = internal standard [echinenone], 6 = (13Z)-ß-carotene, 7 = (all-E)-a-carotene, 8 = (all-E)-rubixanthin, 9 = (all-E)-ß-carotene, 10/11/13 = (Z)-lycopene isomers, 12 = (all-E)-lycopene

Fig. 3. HPLC chromatogram of human plasma extracts before (A) and after (B) supplementation with rosehip purée, C_{30} (250 x 4.6 mm, 5 µm) column (Trentec, Gerlingen, Germany), 1.3 mL min^{-1}, gradient of methanol and MTBE, 17 ± 1 °C, 470 nm

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Study 2
The second intervention study with rosehip purée also showed a significant decrease in contents of lycopene in plasma during the depletion period and a further decrease of lycopene concentrations while supplementing the volunteers with rosehip purée (data not shown).

Discussion
A number of intervention studies has shown a good intestinal absorption of lycopene from tomato products. In contrast, the availability of lycopene was low from raw tomatoes due to protein-carotenoid complexes. These complexes are disrupted by mechanical and thermal processing of tomatoes, improving the availability of lycopene. Increasing the surface of lycopene crystals by reducing the particle size was shown to be an effective processing step to enhance intestinal absorption of lycopene. In addition, presence of tomato oil in oleoresin and partly removal of selective fatty acids affected the intestinal absorption of lycopene, too (Böhm, 2002). Surprisingly, the daily intake of 5 mg lycopene comprised in rosehip purée for four weeks (two separate studies) did not lead to an increase of the contents of lycopene in plasma. In contrast, the concentrations decreased during the intervention period. Heating of the purée before the intervention (study 2) did not change the situation in plasma. Perhaps the ingestion of the purée together with milk proteins (yoghurt, milk) in both studies restrained the availability of lycopene. The content of non-starch polysaccharides in rosehips might be another limiting factor for the absorption of lycopene. Ongoing investigations will determine the contents of these polysaccharides. A recent intervention study with red carrots (carrots with high contents of lycopene) showed that the lycopene in the red carrot is about 44% as bioavailable as that from tomato paste (Horvitz et al., 2004). These results showed that carrot fibre affected lycopene absorption. This is in accordance with a study of Rock and Swendseid (1992). These authors showed that citrus pectin was able to reduce the plasma β-carotene response by more than a half.

Water melons are another good source of lycopene with around 5 mg/100 g. Water melon ranks 5th among the major contributors of lycopene in the U.S. diet. The carotenoid profile is similar to that of tomato. In contrast to tomatoes, water melons are not typically heat treated, a factor that might be expected to limit lycopene bioavailability. Thus, a human intervention study investigated the intestinal absorption of lycopene after consumption of water melon juice compared to tomato juice. The volunteers ingested for three weeks 20 mg/d lycopene comprised in water melon juice or in tomato juice. In addition, 40 mg/d lycopene were tested by giving water melon juice. Lycopene in plasma significantly increased after ingestion of all juices. There were no significant differences in lycopene contents in plasma for all treatments. Thus, lycopene was available in a comparable way from water melon juice and tomato juice. The higher lycopene dose from water melon juice did not lead to a significant higher concentration in plasma compared to the 20 mg doses (Edwards et al., 2003).

Among the indigenous fruits in Vietnam, Momordica cochinchinensis (gac) is a fruit rich in lycopene. Its lycopene concentration was 80.2 mg/100 g edible portion. This fruit is used in Vietnam as provitamin A source due to its content of β-carotene (17.5 mg/100 edible portion). Within an intervention trial (30 d) volunteers daily ingested 20 g gac fruit (16.0 mg lycopene) mixed with 110-120 g cooked rice. The contents of lycopene significantly increased from 0.08 µmol/L to 0.80 µmol/L (Vuong et al., 2002). Thus, lycopene is available from these fruits.

Richelle et al. (2002) investigated the intestinal absorption of lycopene comprised in a food-based formulation "lactolycopene" compared to tomato paste. The formulation contains lycopene entrapped with whey proteins. Healthy subject ingested 25 mg lycopene/d for 8 w. Lycopene concentrations in plasma reached a maximum after 2 weeks of supplementation in both groups and then a plateau was maintained until the end of the treatment. Increases in lycopene contents in plasma (lactolycopene: 0.58 ± 0.13 µmol/L, tomato paste: 0.47 ± 0.07 µmol/L) were not different between supplemented groups (Richelle et al., 2002).

Concluding all human intervention studies with lycopene, heating and processing increases the bioavailability of lycopene. Non-starch polysaccharides, e.g. contained in rosehips, are one limiting factor for the intestinal absorption of lycopene.

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