Selection of the most promising fruit or vegetable for health claim certification

Tomatoes as fresh functional food?

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Tomatoes as fresh functional food?

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Preface

Chronic diseases like cancer and cardiovascular diseases are becoming more and more important in western society. Diet plays an important role in development of chronic diseases, especially high intake of plant compounds is beneficial to health. Many food producers are interested in the addition of health promoting compounds to their products and try to certify this products with a health claim to inform the consumer about the health promoting effects of their products. Our challenge is to assess the possibility of certifying a fresh food product with a health claim. This has never been done before In The Netherlands. With this report, we hope to contribute to a process which in the end will give consumers a choice for fresh functional fruits and vegetables with proven health benefits. Thus we hope to contribute to a healthy society.

The idea to investigate the most suitable fresh fruit or vegetable suitable for certification is commissioned by the consortium BF3 (Best Fresh Functional Food), established in The Netherlands. We performed a literature study to find information about the most promising health compound present in fruits or vegetables or about the most promising fruit or vegetable itself, we searched for information about convincing data from clinical trials, possible mechanisms of actions and pre- and post-harvest conditions. By reviewing this information, we tried to define an advice for the BF3 consortium.

The information in this report is not complete at all points. For example, we did not include labeling procedures of fresh functional foods or consumer behavior in relation to fresh functional foods. We did also not deeply assess the certification procedures in the European Union. However, for now, this report will give an advice on certifications of fresh foods which in the future, have the largest chance of being profitable for the consumer.

Finally, we would like to thank Pascal van Delst of the BF3 consortium for giving us the opportunity to work on this exiting and innovating project, and his readiness to provide us with relevant information. Further, our special gratitude goes to our project coach Mieke Kleijn of Wageningen UR for participating in the project and steering us in the right direction.

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Summary

Scientific evidence for health promoting compounds in fruits and vegetables is increasing. The Best Fresh Functional Food (BF3) consortium aims to certify fresh foods with a health claim. The objective of the project was the identification of a fresh fruit or vegetable that is most likely to be approved for certification for a health claim within limited time. Criteria for certification of a fresh fruit or vegetable were set, regulation and guidelines within the European Union were taken into account. The International Life Science Institute (ILSI) established the Process for the Assessment of Scientific Support for claims on foods (PASSCLAIM). The criteria for the selection of the most promising plant compound are mainly based on the legislation of ILSI. Six different chemical groups (Flavonoids, Tannins, Organosulfur compounds, Terpenoids, Vitamins and Minerals) are studied in this report. Of the flavonoids, quercetin, kaempferol, sylibin, and anthocyanes are studied, of the tannins, proanthocyanidins and ellagitannins are studied, of the vitamins and minerals, selenium and folate are studied, of the organosulfur compounds, glucosinolates and allium compounds and of the terpenoids, monoterpenes and the tetraterpenes, β-carotene, lutein and lycopene are studied. In accordance with the PASSCLAIM, in this study special attention is given to literature on clinical trials and human intervention studies, articles with critical reviews about the assumed health benefits and the more general reviews about the state of current research on phytochemicals in relation to human health.

This research started with a broad literature study on the available scientific evidence for the above mentioned plant compounds. Based on the amount, the quality and the extent of the available clinical trials the most promising compound was selected. Of the mentioned compounds, lycopene is the compound which fits the PASSCLAIM criteria the most. Selection of a phytochemical independent of its matrix has however been proven ineffective. Thus, lycopene within tomatoes is selected for this study as promising candidate for fresh food certification for a health claim. The benefits of tomato intake have mainly been attributed to its lycopene content and lycopene definitely contributes to the health benefits of consumption of tomato products as established by *in vitro*, animal and human studies. Lycopene exhibits antioxidant activities, suppresses cell proliferation and interferes with the growth of cancer cells. Therefore, the conclusion may be drawn that consumption of naturally occurring lycopene-rich fruits and vegetables, particularly processed tomato products containing lycopene, but also the consumption of fresh tomatoes should be encouraged, with positive implications to health.

In the second section of this report, factors influencing the possibility of certifying tomatoes as a fresh functional food are discussed. The mechanism of the protective health effects of lycopene is studied.

It is clearly shown that lycopene is bioavailable in humans. Long term lycopene status, in both blood and tissues, needs to be further explored to investigate the effects of a diet rich in tomatoes.

There are numerous health beneficial mechanisms in which lycopene is involved, either by direct anti-oxidative activity or by enhancing or inhibiting pathways. Synergy may be present between lycopene and for instance α-tocopherol, but the mechanism is still unclear. Until now, no adverse effects of lycopene supplements have been identified.

The effects of tomatoes on human health are diverse and range from several types of cancer to neurodegenerative diseases. Dietary intake of tomatoes and tomato products containing lycopene have been mainly associated with oxidative stress. Increased oxidative stress has been implicated in the incidence of chronic diseases, such as cancer and cardiovascular disease. (Basu and Imrhan 2007)Therefore, a claim on the target function oxidative stress, rather than on a disease, is the most reasonable for the near future. A wide variety of biomarkers for oxidative stress for humans are present, however a conclusive biomarker, satisfying the criteria stated in this report, can not yet be pointed out. Dosage levels are important for a possible claim, but also for future long-term intervention studies. It is however not clear what the ideal lycopene intake for a health effect should be.

Pre- and post-harvest conditions can influence the content and bioavailability of lycopene in the different varieties of tomatoes. The content of lycopene in tomatoes depends on conditions like genetics, environmental factors, the agricultural techniques used, post-harvest storage, ripening, and processing conditions and are reviewed. It would be of great interest to have more data to help to

understand more clearly how these factors affect the accumulation of lycopene during the fruiting period.

In conclusion, there seems to be sufficient evidence to start an application procedure for a health claim at the moment. A claim on the target function oxidative stress, rather than on a disease, is the most reasonable for the near future. A reduction of oxidative stress is assumed to prevent or delay the occurrence of chronic diseases like cardiovascular diseases, cancer, and probably osteoporosis.
The actual EU certification of tomatoes as fresh functional food will need some additional research. A long-term and large valid clinical trial should be performed using fresh tomatoes as intervention and a subject group that is representative for the target group of the claim. Validity of the study used to substantiate the health claim should be based on the legislation of ILSI. In addition, to actually introduce the tomatoes as fresh functional foods on the market, more knowledge about pharmacokinetics, mechanism, synergy, pre- and post-harvest conditions and dose-response relationship is needed/suggested.

Abbreviations

AMI	Acute Myocardial Infarction
BF3	Best Fresh Functional Foods
cv	cultivar
CVD	Cardio Vascular Disease
CYP	Cytochrome P450
DHT	5-α-Dihydrosterone
DW	Dry Weight
EFSA	European Food Safety Authority
FW	Fresh Weight
HDL	High Density Lipoprotein
HPLC	High-Performance Liquid Chromatography
IGF	Insulin-like Growth Factor
IL-6	Interleukin-6
ILSI	International Life Science Institute
LDL	Low Density Lipoprotein
MCP	Methylcyclopropene
NOAEL	No Observed Adverse Effect Level
NPV	Nutritional Prevention of Cancer
OSL	Observed Safe Level
PASSCLAIM	Process for the Assessment of Scientific Support for Claims on Foods
PBMC	Peripheral Blood Mononuclear Cells
PSA	Prostate Specific Antigen
RCT	Randomized Controlled Trial
ROS	Reactive Oxygen Species
TBARS	Thiobarbituric Acid Reactive Substances
ULS	Upper Level of Supplementation
WHO	World Health Organization

Introduction

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years. Plants have served humans valuable components of seasonings, beverages, cosmetics, dyes, and medicines. The World Health Organization (WHO) estimated that about 80% of the world's population rely on traditional medicine for their primary health care. Most of these treatments involve the use of plant extracts or their active components, for example terpenoids. (Wagner, Wang et al. 2004). Scientific evidence for health benefits of fresh plants is increasing. Literature about phytochemicals in fruits and vegetables shows how these products are involved in health promotion.

According to the United States Department of Agriculture, phytochemicals are certain organic components of plants, which are thought to promote human health. Fruits, vegetables, grains, legumes, nuts and teas are rich sources of phytochemicals. For instance tomatoes, cabbage and berries are rich sources of phytochemicals like lycopene, glucosinolates, and anthocyanines respectively, which are expected to benefit human health. Research supports evidence for protective effects on cancer, coronary heart disease, diabetes, hypertension, inflammation, infections, psychotic disease etc. This evidence is mostly based on *in vitro* studies, animal studies and epidemiological studies in humans. However, to prove the efficacy of phytochemicals for human health only evidence from human intervention studies can be used (Dillard and German 2000).
It is essential to take the source and the situation or surrounding wherein a phytochemical originates and develops, the so-called matrix, into account for examining the health effects. This is among others important because of synergetic effects.

Knowledge about the specific effect of phytochemicals within a fruit or vegetable matrix will in time result in the development of fresh functional foods: fresh food products with a high level of a specific compound which has been proven to contribute to human health. The claims on functional foods need strong regulation and a harmonized policy within the whole European Union to prevent the consumer of being mislead. This regulation includes scientific evidence to prove the claim, as established by PASSCLAIM and can be divided into nutrition claims and health claims (Aggett, Antoine et al. 2005).

BF3 (Best Fresh Functional Foods) is a consortium which focuses on the certification of fresh functional foods in Europe for small and medium sized enterprises (SME) in the food and agro sector. The consortium has been recently formed by the enterprises Koppert Cress, Eminent Food and Valstar. The vision of the consortium is to certify fresh functional foods to contribute to health protection of consumers and society. At this moment, no fresh functional food is certified for the European market. The goal of BF3 is to be the first to produce such a certified fresh functional food. Once this first product has been certified, other fruits or vegetables will follow. Therefore a fresh fruit or vegetable that is most likely to get this certification in the near future has to be identified.

Objective

The main objective of this literature study is the identification of a fresh fruit or vegetable that is producible in the Netherlands and is most likely to be approved for certification for a health claim within limited time.

The following sub-objectives are formulated to answer the main objective:

- What are the criteria for scientific evidence in relation to certification of fresh food products?
- Which known phytochemicals present in fresh fruit or vegetables have the strongest scientific evidence for health promoting benefits in humans?
- Which fresh fruit or vegetable matrix is a suitable source for the most promising phytochemical?
- in which way do the following factors influence the possibility of certifying a fresh food product:
    - Bio-availability
    - Genetic variety
    - Environmental conditions
    - Synergistic effects
    - Adverse effects
    - Dose-response relationship
    - Storage and processing

Approach

To reach the objectives an initial broad literature study on the available scientific evidence is conducted. For this, the general databases of scientific literature are searched with programs like ISI Web of Knowledge, WebSpirs, PubMed and Google Scholar. These programs include among others, databases like “Medline” and “CAB abstracts” which are specifically focused on the topics addressed in this study.

In accordance with PASSCLAIM, in this study special attention is given to literature on clinical trials and human intervention studies, articles with critical reviews about the assumed health benefits and the more general reviews about the state of current research of phytochemicals in relation to human health. This part of the study will yield a number of phytochemicals, present in fresh fruits or vegetables which are believed to have a beneficial effect on human health. Those phytochemicals will be shortly reviewed and related human intervention studies will be shown in tables. After that, all those phytochemicals will be discussed according to their potential for certification as a fresh functional food. Argumentation for certification potentials will be based on beforehand formulated criteria. After selecting the most promising phytochemical, the most suitable fresh fruit or vegetable matrix, producible in the Netherlands, for this compound will be identified. Subsequently, pre-harvest, post-harvest, mechanism, dose-response relationship, bioavailability and adverse effects of the chosen fresh product will be discussed. Substantiation of a health claim for the chosen product, based on the criteria of the PASSCLAIM of the International Life Science Institute (ILSI), will be addressed. Finally, advise for further research, needed for a health claim, will be discussed.

1 Criteria for certification of a fresh functional fruits or vegetables

To assess whether a fresh food product is suitable to be certified as a functional food, regulation and guidelines are formulated within the European Union. Regulation is formulated by the European Food Safety Authority (EFSA) as regulation (EC) No. 1924/2006 (Aggett, Antoine et al. 2005) and a guideline for assessing the available literature supporting the food claim, is given by the European branch of the International Life Science Institute (ILSI). ILSI established the Process for the Assessment of Scientific Support for claims on foods (PASSCLAIM) (Aggett, Antoine et al. 2005). In this section, criteria for the substantiation and approval of a health claim will be addressed. These criteria are mainly based on the legislation of ILSI for health claims. In addition, criteria needed for the selection of the most promising phytochemical and matrix that is most likely to be approved for certification for a health claim within limited time, are formulated.

Figure 1 shows the hierarchy of evidence from different types of studies to support claims. The types of studies required to support a health claim, beginning with the most important, are randomized controlled intervention studies (RCTs), less well-controlled types of intervention studies, prospective observational human studies, retrospective observational human studies, animal studies, and *in vitro* cellular studies (Ashwell 2002). Critical reviews play a large role in the substantiation of a health claim.

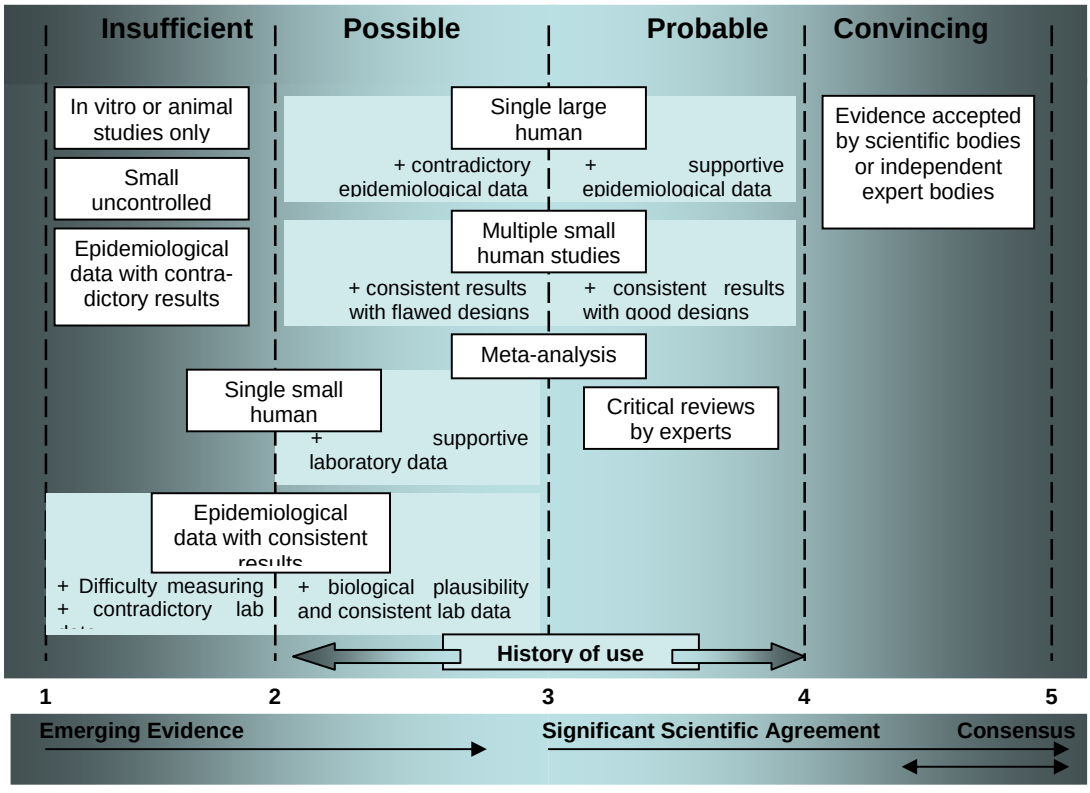

Figure 1. The hierarchy of evidence from different types of studies to support claims (from TNO presentation)

The validity of studies used to substantiate a health claim should be ensured. Validity of studies is improved if (Ashwell 2002):

- The subjects are representative of the target group for the claim.
- The subjects consume a reasonable amount of the food or food component in question at a reasonable frequency, consistent with realistic consumption patterns.
- The study is large enough to demonstrate the proposed beneficial effect. The desirable size for a study can be assessed using standard formulae for power analysis.
- The duration of the study is long enough to justify any implication of the claim that a beneficial effect is a long-term rather than a short-term effect.
- The outcomes are measured properly and according to standard procedures.
- The outcomes are identical or similar to those of the claimed effect. If the claim refers, for example, to a risk factor for a disease, then at least some of the studies used to substantiate the claim should involve measuring that risk factor.
- Possible confounding variables are taken into account. In a study of the association between a food or food component and a beneficial effect, confounding can occur when the studied population is simultaneously exposed to something else (for example, an unavoidable change in total fat intake when looking for an effect of increasing the n-3 PUFA content of the diet) that could be associated with the proposed cause and effect.

Claims should be based on evidence related to markers that are linked to clearly defined and measurable outcomes and consistently modulated by the particular food component in rigorously controlled studies. Figure 2 (Ashwell 2002) shows the relation between the type of markers and the type of claim. If evidence is based on a marker of enhanced function, such as changes in body fluids or tissues, or levels of metabolite, then an enhanced function claim can be made. If evidence is based on a marker of reduced risk of disease, such as the measurement of a biological process that relates directly to the endpoint, then only in this case can a reduction of disease risk claim be made. Markers should be feasible (i.e. measurable in easily accessible material or obtainable using ethical or minimally invasive methodology), valid, reproducible, sensitive and specific, plausibly linked to the phenomena involved in the biological process being studied and should represent relatively immediate outcomes that can be used to assess interventions in a reasonable timescale. In addition, markers should be rigorously internally validated to establish sensitivity, specificity and reproducibility in different centers. The effect measured by the selected marker should be physiologically and statistically significant. Moreover, markers should be generally accepted in the scientific field as valid in relation to the function and/or disease risk (Ashwell 2002).

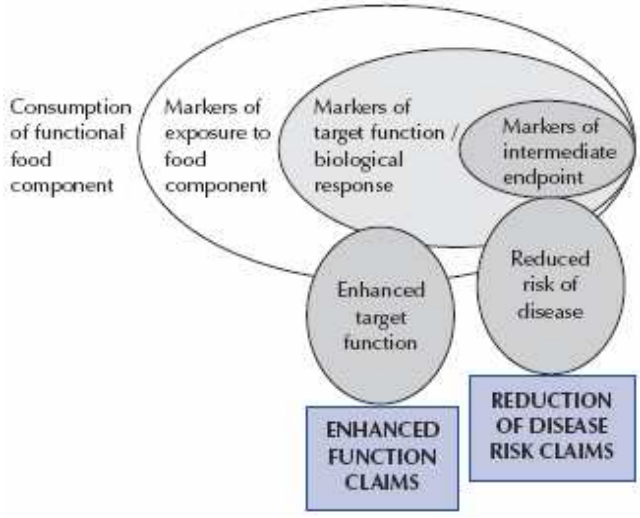

Figure 2. Functional foods: a proposal for a scientific basis for claims (from FUFOSE Consensus Document) (Ashwell 2002)

For the purpose of this paper, the following table is created to aim the selection of the most promising phytochemical and matrix that is most likely to be approved for certification for a health claim within limited time:

Table 1. Summary of available clinical trial on phytochemicals and matrixes

Phytochemical/food product: Database(s): Search: Limits:							
Author, year, location; intervention length and research design	Intervention (dose, chemical form)	Subjects (age, gender, special properties/disease)	Study group		Bio-availability (if information available)	(Intermediate) endpoints	Notable results
			Intervention group (n)	Control group (n)			
…							

This table will be used to summarize available information on all phytochemicals and matrixes that are investigated in this paper. Only RCTs, not older than 10 years, will be included in the tables. The final selected phytochemical and/or matrix will be the one that is studied the most within valid RCTs concerning the phytochemical and/or matrix in relation to human health, and resulted in the most positive (statistically significant) results. In addition, interventions performed with the fruit (product) or vegetable (product) containing the phytochemical, instead of phytochemical supplementations, are preferred. Moreover, the fruit or vegetable matrix that contains the assumed health promoting compound in question must be producible in the Netherlands.

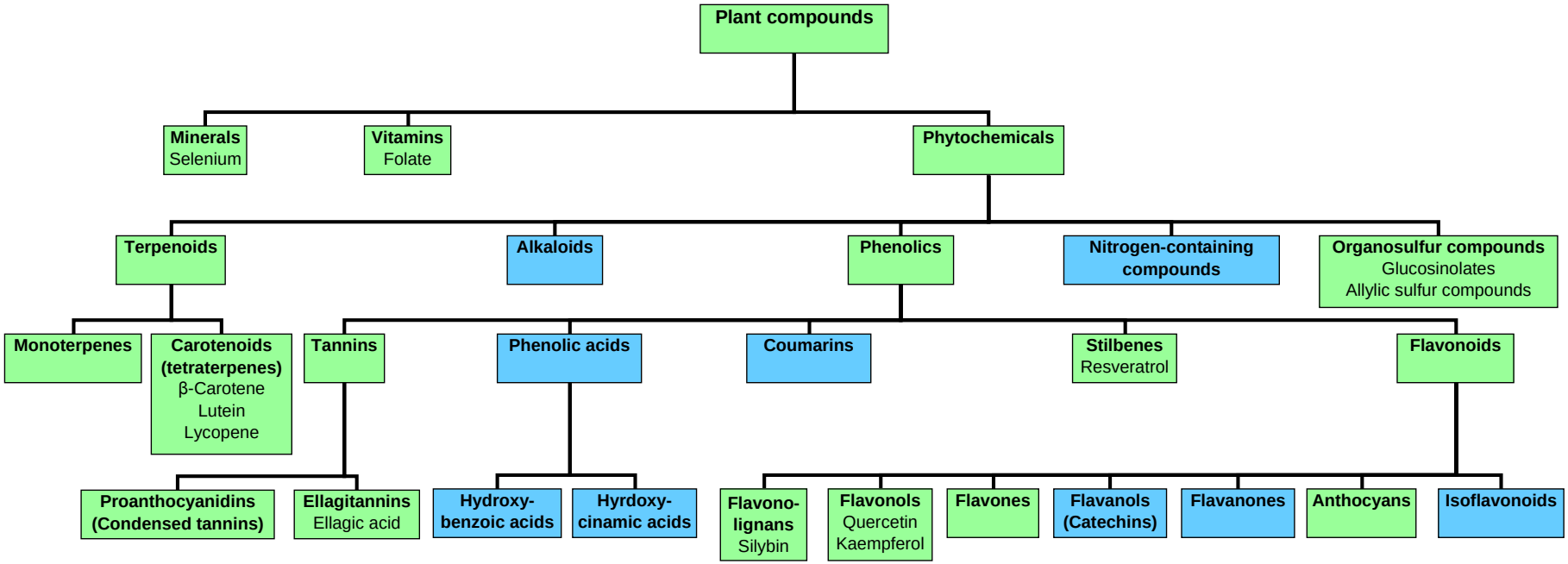

Figure 3. Overview of the different major plant compounds (green = health promoting compounds studied in this paper)

2 Health promoting plant compounds

This chapter tries to answer the following questions:

- Which known phytochemicals present in fresh fruits or vegetables have the strongest scientific evidence for health promoting benefits in humans?
- Which fresh fruit or vegetable matrix is a suitable source for the most promising phytochemical?

Therefore a selection of different compounds of six different chemical groups (Flavonoids, Tannins, Organosulfur compounds, Terpenoids, Vitamins and minerals) will be studied in this chapter. Figure 3 shows an overview of the different plant compounds and the ones in the context of this study are highlighted in green. Only vegetables or fruits that are producible in the Netherlands are studied.
In accordance with PASSCLAIM, special attention is given to literature on clinical trials and human intervention studies, articles with critical reviews about the assumed health benefits and the more general reviews about the state of current research of the phytochemical in relation to health. For all phytochemicals and matrices studied, tables with a summary of available clinical trials are included in Appendices I-IX . The table design is described earlier in the criteria chapter.
For the selection of the most promising phytochemical and matrix that is most likely to be approved for certification for a health claim within limited time, earlier mentioned PASSCLAIM criteria were applied. The selected phytochemical and/or matrix is the one which is studied the most within valid RCTs concerning the phytochemical and/or matrix in relation to human health, and resulted in the most positive (statistically significant) results.

2.1 Flavonoids

An important criteria for a fruit or vegetable to be chosen for further research in this report is the possibility of production in The Netherlands. Therefore, in this literature research, isoflavones, flavanols and flavanones have been excluded. Isoflavones are mainly present in soy products, flavanols in tea products, and flavanones in citrus fruits. All of those products are not produced in The Netherlands.

2.1.1 Quercetin

Quercetin can be found in fruits and vegetables at concentrations of for example 50 mg/kg fresh edible part for apples and 300 mg/kg fresh edible part for onions (Hertog, Hollman et al. 1992). These data show onions are fresh products with the highest quercetin concentrations among fruits and vegetables. Quercetin is a natural antioxidant, able to transform into other antioxidants like kaempferol and isorhamnetin *in vivo*. The absorption of quercetin takes place after conjugation or glucosidase reactions in the small intestine (Hollman and Katan 1997).

Evidence for health promoting effects derived from animal studies includes increased resistance to copper sulfate-induced lipid peroxidation, antihypertensive properties in hypertensive rats (but not in normotensive rats), and anticarcinogenic properties, due to the antioxidant activity, which was also seen in *in vitro* models (Perez-Vizcaino, Duarte et al. 2006). Although animal studies show positive results, the evidence from epidemiological studies and human intervention studies is not very convincing. Nevertheless, epidemiological data show a protective effect against oesophageal and stomach cancer after onion consumption (Gao, Takezaki et al. 1999), a reduction in brain cancer risk due to onion as part of the diet (Hu, La Vecchia et al. 1999), and a reduction in breast cancer risk after consumption of onions, garlic and fibers. In contrast to these beneficial effects on breast cancer risk, a large prospective study showed no relation between breast cancer and onions (Dorant, Vandenbrandt et al. 1994). Whether the positive effect in these studies is due to the health effects of quercetin per se, other compounds or synergetic effects is not known. Clinical trials with quercetin supplementation do not show a beneficial effect on carcinogenesis or cardiovascular disease (CVD) risk factors. Quercetin supplementation does however result in elevated plasma antioxidant levels, but does not reduce lipid oxidation or DNA damage, biomarkers for cancer and CVD (Conquer, Maiani et al. 1998; Boyle, Dobson et al. 2000). In addition to a clinical trial with quercetin supplementation, Boyle *et al.* set up a study involving the consumption of onions in addition to a low flavonoid diet. Onion consumption increased plasma quercetin glycoside levels and increased resistance of lymphocyte DNA to breakage (Boyle, Dobson et al. 2000).

Positive effects were seen in endurance exercise performance in professional cyclists after a cocktail containing quercetin compared to the same cocktail without quercetin (MacRae and Mefferd 2006). This study was performed on only 12 cyclists. Therefore, further research concerning endurance performance is recommended. Positive results were also seen in dyalisis patients after renal transplantation. In patients who received a capsule containing curcumin and quercetin, renal function was improved, compared to the control group (Shoskes, Lapierre et al. 2006).The positive effect is possibly due to the administration of curcumin, because this flavonoid was also present in the capsule, or possibly due to synergetic effects.

A study with patients suffering from persistent allergic rhinitis showed a decrease in their complaints after receiving apple juice. This effect is possibly due to proanthocyanidines present in apples and not to quercetin per se (Enomoto, Nagasako-Akazome et al. 2006). A diet high in quercetin due to a different agronomical production method showed an improved antioxidant status and an improved glutathione enzyme status. However, this study was performed in a small group (Grinder-Pedersen, Rasmussen et al. 2003).

In conclusion, the health promoting benefits of quercetin are questionable. Products containing quercetin have some antioxidative effects, but whether this effect is only due to quercetin is unknown. Quercetin supplementation solely showed almost no effect. The health promoting effects of quercetin

in onions are not fully understood and more research on this vegetable in relation to quercetin and synergy with other flavonoids is recommended.

2.1.2 Kaempferol

Kaempferol is the second most occurring flavonol present in fruits and vegetables in The Netherlands (Hollman and Katan 1997). Moreover, this flavonol is closely related to quercetin. *In vitro* studies suggest an inhibitory effect of kaempferol on the procarcinogenic gene CYP (cytochrome P450) through antagonizing TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) effects, a potent carcinogenic compound (Moon, Wang et al. 2006).
A newly developed insight in mechanism is the possibility of increasing energy expenditure in muscle cells, which gives therapeutic opportunities for patients with metabolic problems (da-Silva, Harney et al. 2007). Furthermore, a Japanese study showed weight controlling and plasma triglyceride lowering effect *in vivo* in male Wistar rats (Yu, Shun et al. 2006).
Information about the beneficial properties of kaempferol in animal studies is scarce and not convincing. An epidemiological study on kaempferol intake showed no relation with colon cancer (Lin, Zhang et al. 2006). Further research in animal studies should be focused on the anti-obesity effects. In case of positive results, further research on humans have to be performed.

2.1.3 Silybin

Silybin from the silymarin complex is a natural antioxidant derived from milk thistle (Dutch: Mariadistel) and artichoke. For 2000 years, this plant extract has been used as a liver protective extract and for problems with the gastro-intestinal tract. However, the mechanism for this protective effect is unclear. Moreover, evidence is not fully established due to the scientific quality of the available studies (Gazak, Walterova et al. 2007). Besides the probable protective effects for the liver and GI-tract, interest has risen for the anticarcinogenic properties of silybin. Cell studies have shown that silybin inhibits growth of cancer cells including prostate cancer cells by modulating pathways involved in cell growth and cell cycle progression. Furthermore silybin can act as a modulator on different signaling pathways in the cell (Gazak, Walterova et al. 2007). Several animal studies showed an anticarcinogenic effect in mice with prostate cancer (Sharma, Agarwal et al. 2001), urinary bladder cancer (Vinh, Sugie et al. 2002), skin cancer (Agarwal, Katiyar et al. 1994; Lahiri-Chatterjee, Katiyar et al. 1999; Zhao, Lahiri-Chatterjee et al. 2000) and colon cancer in rats (Kohno, Tanaka et al. 2002). Silybin seems to have a protective effect on the gastro-intestinal tract. It stimulates pancreas recovery after necrosis of insuline-producing β-cells in rats through antioxidative activities and/or through an increase in pancreatic glutathione concentrations (Soto, Recoba et al. 2003).
The positive effect on the pancreas is tested in subjects with type II diabetes mellitus in a double-blind, randomized placebo-controlled clinical trial. Intervention with a silymarin capsule significantly lowered fasting blood glucose, HbA1c (glycosylated hemoglobin). Silymarin also significantly lowered total cholesterol, plasma low-density-lipoprotein (LDL), plasma triglycerides and liverenzymes SGOT (serum glutamic oxaloacetic transaminase) and SGPT (serum glutamic pyruvic transaminase). In the treatment group as well as in the placebo group plasma high-density-lipoprotein (HDL) was lowered significantly (H. Fallah Huseini 2006).
In addition to the above mentioned studies, clinical trials of silybin on prostate cancer are in progress. The overall advice is to focus on studies with vegetables containing high amounts of silymarin and assess the bioavailability and the health effects in clinical trials, especially focused on lipid metabolism, liver functioning and diabetes.

In conclusion, the best known and probably most potent flavonoid is quercetin.
Important in the research on health effects by flavonoids is to understand that not the flavonoid per se has beneficial health effects, but that the mix of flavonoids occurring in the food products has the beneficial effect. Possibly, this effect is due to synergism between several flavonoids or phytochemicals. Another factor can be that the bioavailability of the flavonoid differs in the food

product or during digestion. For these reasons, studies should focus on food products and not on the flavonoids per se.

2.1.4 Anthocyans

Anthocyans are flavanoids which are known for the red, blue and purple color they give to flowers, fruits and vegetables. The term anthocyan covers both the terms 'anthocyanin', the glycoside form, and 'anthocyanidin', its aglycon (the non-sugar compound remaining after replacement of the glycosyl group from a glycoside by a hydrogen atom).

Relatively high concentrations of anthocyanins are found in the diet compared to other flavanoids. The highest concentrations of anthocyanins in berries are found in bilberry (*Vaccinium myrtillus*), chokeberry (*Aronia medik*) and crowberry (*Empetrum nigrum*). In other fruits anthocyanins can be found in cherry, red grape and dark plum. Vegetables rich in anthocyanins are red cabbage, red radish and red onions. It should be noted that even in these 'top' fruits and vegetable anthocyanin content is as much as 8-40 times lower than the highest content found in berries (bilberry: 611.3 mg/100 g fresh weight) (Koponen, Happonen et al. 2007).

The six most common anthocyans in the edible parts of plants are cyanidin, pelargonidin, peonidin, delphinidin, petunidin and malvidin (Kong, Chia et al. 2003; Koponen, Happonen et al. 2007). Whereas most investigated foods just contain cyanidin, the most divers profile of anthocyans can be found in bilberry, blueberry and bog whortleberry (*Vaccinium uliginosum*) (Koponen, Happonen et al. 2007).

In vitro studies revealed that anthocyanidins show greater potency to inhibit neoplastic cell survival than anthocyanins. Delphinidin was found to posses the highest growth-inhibitory activity. Anthocyans also interfere with tumor promotion or progression mediated by cyclooxygenase (COX), tyrosine kinases and phosphodiesterases. The rank order of potency of anthocyans with respect interference in these systems can be considerably different. For example, delphinidin and cyanidin are the most potent inhibitors of a certain tyrosine kinase, but are least potent in inhibiting phosphodiesterase activity. Anthocyans also play a role in interruption of the cell cycle, and therefore in apoptosis and antiproliferation of the cell. Furthermore different assays showed that anthocyans are potent antioxidants (Cooke, Steward et al. 2005).

Several animal studies show positive cancer chemopreventive properties of anthocyans (Cooke, Steward et al. 2005).

However, the fate of anthocyans in the human body is poorly investigated compared to other flavanoids. In general most studies have found unmetabolized anthocyanin glycosides in human blood and urine. It was therefore suggested that anthocyanins are not metabolized before release into the systemic circulation. However recent evidence shows that 68-80% of anthocyanins as metabolized derivatives in human urine (Kay 2006). It is also likely that anthocyanins are extensively metabolized to phenolic acids by the colonic microflora (Kay 2006).

Epidemiological evidence on anthocyans is not abundant, but slightly hints at a anti-carcinogenic effects and reduction of the risk on coronary heart disease (Cooke, Steward et al. 2005).

The few clinical trials with anthocyans are mostly on night vision and oxidative DNA-damage. With respect to night vision study results are not unequivocal. One randomized controlled double-blind cross-over study reported no effect of a 25%-anthocyanoside bilberry extract on night visual acuity and contrast sensitivity (Muth, Laurent et al. 2000). Another trial indicated improvement of dark adaptation and subjective asthenopia symptoms after administration of black current anthocyanoside (Nakaishi, Matsumoto et al. 2000).

Positive results are found in a study on the effect of 85%- anthocyanoside oligomer tablets on the improvement of subjective symptoms and objective contrast sensitivity in myopia subjects with asthenopia (Lee, Lee et al. 2007).

(Moller, Loft et al. 2004) performed an intervention study on the effects of blackcurrant juice and specifically blackcurrant anthocyanins on the steady state level of oxidative DNA damage in mononuclear blood cells. Subjects completed a 3-week controlled parallel intervention study with three groups randomized to supplementation with blackcurrant juice, anthocyanin drink, or a control drink. It was concluded that this study shows that even large amounts of dietary antioxidants did not decrease

the already low steady state levels of oxidative DNA damage in healthy adequately nourished humans (Moller, Loft et al. 2004). Another study did show a significant decrease in oxidative DNA-damage and an increase in reduced glutathione and glutathione status, though lipid peroxidation, DNA-binding activity of nuclear factor-κβ and plasma carotenoid/α-tocopherol levels were not significantly altered (Weisel, Baum et al.). These results can however not be attributed to either anthocyans, nor to a specific fruit, since the intervention consisted of a mixed fruit juice. Moreover, this juice was not exactly the same for placebo and control group and the intervention of both groups did not take place at the same time.

More research is needed on bioavailability, especially on metabolites formed and their bioactive properties. This should include research on the role of the food matrix in absorption of the anthocyans. In addition, attention should be paid to the different anthocyans and/or their metabolites. They may have different functions in the body, and therefore be of different interest. *In vivo* studies should further investigate whether exposure in both time and concentration is sufficient for exertion of anticarcinogenic effects.

2.2 Stilbenes

2.2.1 Resveratrol

In addition to our focus on flavonoids and tannins, resveratrol, a stilbene, seems to be important as well. Resveratrol is found in grapes (and red wine), peanuts, blueberries and bilberries. The latter however, contains very low amounts of resveratrol. Studies with cell lines and animal studies (rodents) show an anticarcinogenic effect of resveratrol on all three stages of carcinogenesis. However, no human data have been published yet (Baur and Sinclair 2006).
Future research should be on fruits rich in resveratrol. Especially the skin and seeds of grapes contain resveratrol. These studies need to focus on bioavailability of resveratrol from fruits and on potential anticarcinogenic properties.

2.3 Tannins

2.3.1 Proanthocyanidins

Proanthocyanidins, also called condensed tannins, are the second most abundant group of natural phenolics after lignin (Prior and Gu; Rasmussen, Frederiksen et al.), and responsible for the astringent character of the foods which contain them (Rasmussen, Frederiksen et al.). Some important sources of proanthocyanidins are chocolate, hazelnut, some berries, apples, grapes and cinnamon (Beecher 2004; Prior and Gu 2005; Rasmussen, Frederiksen et al. 2005). In this section only the effect of anthocyanidins in berries, grapes and apples is discussed.

In vitro studies show that proanthocyanidins have an effect on several mechanisms involved in cancer development and atherosclerosis (Beecher 2004; Prior and Gu 2005; Rasmussen, Frederiksen et al. 2005). Animal studies seem to support these findings, especially in the case of atherosclerosis (Beecher 2004; Prior and Gu 2005; Rasmussen, Frederiksen et al. 2005).

Increasing chain length of proanthocyanidins has shown to enhance anti-oxidant activity (Rasmussen, Frederiksen et al. 2005), however only low molecular weight proanthocyanidin oligomers are absorbed in the GI tract (Beecher 2004; Rasmussen, Frederiksen et al. 2005). Though *in vitro* gastric juice degraded oligomers, several studies have found that this does not occur *in vivo* (Rasmussen, Frederiksen et al. 2005). Instead proanthocyanidins are degraded to small phenolic acids by microflora in cecum and large intestine (Beecher; Rasmussen, Frederiksen et al.). It therefore appears that anthocyanidins only exert local effects in the GI-tract or effects that are mainly mediated by small phenolic acids (Rasmussen, Frederiksen et al. 2005).

Human intervention studies and clinical trials on proanthocyanidins of foods grown in Europe are scarce and focus on different topics. In a study on cranberry proanthocyanidins it was suggested that presence of the A-type linkage in these proanthocyanidins may enhance both *in vitro* and urinary bacterial anti-adhesion activities and aid in maintaining urinary tract health (Howell, Reed et al. 2005). In a double-blind randomized placebo controlled cross-over study among male smokers supplemented with capsules containing grape procyanidin extracts, positive trends were found on several indexes of LDL-oxidation, though only lag phase and reduction of thiobarbituric acid reactive substances (TBARS) were statistically significant (Vigna, Costantini et al. 2003). An open design study with orally administered 81% proanthocyanidin grape seeds extract showed positive results on chloasma (acquired hypermelanosis). However, in this study no placebo group was used. Another study failed to show efficacy of orally-administered grape seed proanthocyanidin extract in patients with breast induration following radiotherapy for breast cancer (Brooker, Martin et al. 2006).

In these studies proanthocyanidins were not tested as such, but rather as a part of grape seed extracts. Therefore non of the effects found can be specifically attributed to proanthocyanidins. Moreover, no proanthocyanidin-rich fresh fruits or derived products were tested in these studies. Studying fresh products is essential for the investigation of health effects of fresh foods.

Therefore more research on this area needs to be done. The degradation of proanthocyanidins by the colonic microflora should also be further investigated, both *in vivo* and *in vitro*. Also local effects that proanthocyanidins might have in the GI-tract should be investigated. It should be stressed that proanthocyanidins are not an homogenous group of compounds, i.e., their structure may vary, dependent on their sources. This also implies that effects found for one (source of) proanthocyanidin cannot be extrapolated to other (sources of) proanthocyanidins per se.

2.3.2 Ellagitannins

Ellagitannins are the complex derivatives of ellagic acid, a compound that is released after hydrolysis of ellagitannins in the GI-tract (Koponen, Happonen *et al.* 2007). Ellagic acid is a phytochemical which has shown powerful antioxidant activity in several *in vitro* studies (Hannum 2004). Together with ellagitannins, ellagic acid is mainly present in small soft fruits like raspberries, blackberries, cloudberries and strawberries. It has also been detected in pomegranate, walnuts and oak-aged wines

(Clifford and Scalbert 2000; Koponen, Happonen et al. 2007). The focus of this section will be on ellagic acid in relation to caneberries and strawberries.

In vitro studies have shown that ellagic acid has anticancer effects. There are several possible mechanisms by which ellagic acid may exert these effects: decreasing metabolic activation of certain carcinogens by inhibition of CYPs, inducing phase II detoxifying enzymes, scavenging free oxygen radicals and reactive carcinogen metabolites, binding to DNA which prevents attacks by carcinogens, suppressing of ornithine decarboxylase which reduces the neoplastic capability of cells, and inhibiting DNA topiomerases I and II, which are needed for cell proliferation (Hannum 2004). Besides it anticancer effects, antimicrobial activity of ellagic acid is reported as well (Clifford and Scalbert 2000).

The effect of ellagic acid and ellagitannins on carcinogenesis in animal studies is not unequivocal. Some studies show inhibition of tumor initiation and progression, while others show no effect (Clifford and Scalbert 2000).

Although the results of the *in vitro* studies are promising and positive effects on carcinogenesis have been found in some animal studies, ellagic acid appears to be poorly bioavailable in humans (Cerdá, Tomás-Barberán et al. 2005). This is found to be due to conversion of ellagic acid into the bioavailable compounds urolothin A and B by the colonic microflora (Cerdá, Espín et al. 2004; Cerda, Periago et al. 2005; Cerdá, Tomás-Barberán et al. 2005). However in one study with freeze-dried black raspberries the presence of ellagic acid was reported (Stoner, Sardo et al. 2005).

There are no epidemiological studies supporting the beneficial effects of ellagic acid, though this is rather due to absence of these studies than their outcomes.

Hardly any clinical trials can be found on the supposed health effects of ellagic acid. One clinical trail investigated the effect of ellagic acid supplementation (180 mg/day) in patients with hormone refractory prostate cancer (HRPC) on standard chemotherapy using vinorelbine and estramustine phosphate. A reduction in systemic toxicity, statistically significant for neutropenia, associated with better results in term of objective response rate, individual clinical response, and biochemical response were observed in the intervention group compared with the control group. On the other hand no significant difference in overall survival and progression-free survival was detected between both groups (Falsaperla, Morgia et al. 2005).

Since there are only few data available on the possible health promoting effects of ellagic acid in humans, future research should focus on clinical trials and human intervention studies. Three main areas are of special interest: the effect of ellagic acid on the gastro-intestinal tract; the function of (bioavailable) ellagic acid metabolites produced by colonic microflora, both *in vitro* and *in vivo*; and the further investigation of the bioavailability of ellagic acid.

2.4 Minerals and vitamins

2.4.1 Selenium

Epidemiological data showed beneficial effects of selenium intake on cancer incidence, especially lung cancer, esophageal cancer, gastric-cardia cancer and prostate cancer incidence and also on the risk for colo-rectal cancer (Rayman 2005). The epidemiological evidence is supported by many studies in animals and cells lines. The hypotheses for mechanisms of actions are reviewed extensively. It is suggested that selenium causes irreversible apoptosis with DNA strand breaks, cell cycle arrest and/or apoptosis independent of DNA strand breaks. Moreover changes in the mitogen-activated cell signaling pathway and inhibition of angiogenesis (Finley 2005). Clinical trials with selenium seem promising for cancer prevention. The NPV (Nutritional Prevention of Cancer) Trial gives the strongest evidence for the secondary endpoints all-cause mortality and total cancer mortality, total cancer incidence, and the incidences of lung, and colorectal cancers and especially for prostate cancer (Clark, Combs et al. 1996). The Linxian trials (Blot, Li et al. 1995) showed significant reductions in total mortality and cancer mortality, especially esophagus and stomach cancer. These two studies have both a long intervention time and a large intervention group. Not all the study groups consist of healthy subjects. There are human intervention studies with only healthy subjects which give positive results, but those studies are small and some showed no or no significant effect on cancer biomarkers. The daily dose of selenium in studies with positive outcomes was 200 µg. There were no clinical trials about selenium in relation to cardiovascular disease endpoints found. All studies with selenium were with supplements or selenized yeast, not with Se-enhanced vegetable. Important clinical trials are underway like the SELECT trial (Klein 2004). However, also this intervention is conducted with selenium supplements only.

There are no human intervention studies with selenium in vegetables conducted. In conclusion, there is more research needed on the enhancement of selenium within vegetables. Some studies showed a decrease in glucosinolates in Se-enhanced broccoli (Finley, Sigrid-Keck et al. 2005). Thus there is more research needed on these synergistic effects in vegetables with other compounds.

More knowledge about these foods would be useful for future clinical trials with selenium in vegetables. Positive outcomes of clinical trials with Se-enhanced vegetables in healthy subjects would provide strong evidence for a health claim.

2.4.2 Folate

Folate's role in preventing neural tube defects is well accepted. This has been translated into major public health intervention programs for women in the reproductive age. Folate from vegetables and fruits may also have other beneficial health effects, reflected in epidemiological reviews.

From epidemiological studies can be concluded that there is an inverse relationship between plasma folate level and homocysteine. Homocysteine is a risk factor for cardiovascular disease (Eikelboom, Lonn et al. 1999).

Observational studies evaluated the relationship between folate and colorectal and cervical cancer. Epidemiological evidence about reducing the risk of colorectal adenoma and cancer is rising. Especially, effects are seen among subjects consuming high amounts of alcohol. In many of these studies folate was consumed in supplements, in such amounts that it is unlikely to be reached by dietary folate (Rampersaud, Bailey et al. 2002),(Giovannucci 2002).

Dietary folate potentially influences carcinogenesis because of DNA methylation, synthesis and repair (Giovannucci 2002).

Elevated homocysteine is a risk factor for cardiovascular disease. Homocysteine is remethylated in a process that requires methionine synthase, vitamin B12 as a cofactor, and methyltetrahydrofolate as a cosubstrate. This pathway requires an adequate supply of folic acid (Eikelboom, Lonn et al. 1999).

A human dietary controlled trial investigated the dietary folate from vegetables and citrus fruit consumption on the decrease of plasma homocysteine concentrations. The plasma homocysteine

concentrations decreased significantly in the dietary folate group (Brouwer, van Dusseldorp et al. 1999). Another clinical trial investigated the effect of folate on endothelial function of patients after an acute myocardial infarction (AMI). There was a significant improvement in endothelial function in post-AMI patients, independent from homocysteine status. The dose of supplement was 10 mg daily, which is very high. The plasma folate levels achieved in this study cannot be obtained by dietary fortification with folic acid (Moens, Claeys et al. 2007).
There are a lot of human intervention studies conducted with folate and the effect on CVD and cancer, but almost all with very high doses from supplements.
Positive and significant effects are currently almost always seen with high doses of folate in supplements. Large clinical trials are needed with fresh fruits and vegetables to investigate the effects on cancer biomarkers and risk factors as homocysteine.

2.5 Organosulfur compounds

2.5.1 Glucosinolates (Brassica vegetables)

Glucosinolates are the most studied bioactive components of the Brassica family. Brassica vegetables include broccoli, Brussels sprouts, cauliflower, kale, cabbage and other greens.
The intake of Brassica vegetables and the effect on cancer is reviewed extensively. The cohort studies and case control studies showed an inverse relation between Brassica consumption and risk at cancer at various sites (van Poppel, Verhoeven et al. 1999). The epidemiological literature provides modest support for the hypothesis that high intake of Brassica vegetables reduces prostate cancer risk (Kristal and Lampe 2002).
Glucosinolates are not bioactive in humans, they are enzymatically hydrolysed to isothiocyanates, nitriles or thiocyanates (Keck 2004).
In *vitro* and in *vivo* studies have reported that isothiocyanates affect many steps of cancer development including modulation of phase I and II detoxification enzymes, functioning as a direct or as an indirect antioxidant by phase II enzyme induction, modulating cell signaling, induction of apoptosis, control of the cell cycle and reduction of helicobacter infections (Finley 2005).
Human intervention studies are limited in number and scope. Most intervention studies examined bioavailability of glucosinolates from Brassica vegetables. Two very small studies showed reduction of DNA damage due to consumption of Brussels sprouts (Verhagen, 1997 #9}, (Bogaards 1994). Another study with Brassica vegetables in general, showed significant decrease in the oxidative stress biomarker in the intervention group. In these studies 200-300 grams cooked vegetables were consumed daily. One larger intervention study with drinking hot infusions of 3-day-old broccoli sprouts resulted in no significant differences between intervention arms, although there were large inter-individual differences in bioavailability (Kensler, Chen et al. 2005).
The amount of clinical trials is limited and the number of subjects is often small. There are no significant positive results in large human intervention studies at all. In the future, more studies are needed, especially large randomized, controlled human intervention trials for a possible health claim on a Brassica vegetable. There is more known about the pre- and post- harvest conditions of glucosinolates in vegetables, but also here more research is needed.

2.5.2 Allium compounds (garlic and onion)

Epidemiological studies show an inverse correlation between garlic consumption and progression of cardiovascular disease (Rahman and Lowe 2006). Furthermore, evidence from other epidemiological studies suggest a preventive effect of garlic consumption in cancer, especially stomach and colorectal cancer (Fleischauer and Arab 2001). The sulfur-containing compounds allicins are believed to be responsible for the health benefits. These sulfur compounds are also present in onions and are produced for the protection of the so-called Allium plants themselves (Ariga and Seki 2006).
Numerous *in vitro* and animal studies indicate that garlic induces phase II detoxification enzymes, and contains antioxidant-, antiproliferating-, and differentiation inducing effects (Ariga and Seki 2006). As a result, garlic seems to enhance carcinogen-neutralizing activity, reduce cholesterol, inhibit platelet aggregation, reduce blood pressure, and increase antioxidant status (Ariga and Seki 2006; Rahman and Lowe 2006). A large number of clinical trials concerning garlic and its health beneficial effects have been performed. In the last five years, ten important RCTs and cross-over RCTs have been published. Most of the studies show no beneficial health effect (Peleg, Hershcovici et al. 2003; Tanamai, Veeramanomai et al. 2004; Turner, Mølgaard et al. 2004; van Doorn, Santo et al. 2006; Zhang, Gail et al. 2006; Gardner, Lawson et al. 2007). All of the studies used garlic extract and/or garlic powder supplementation as intervention. Two of them specifically mentioned the use of enteric-coated tablets (Tanamai, Veeramanomai et al. 2004; Kojuri, Vosoughi et al. 2007) for an improved bioavailability. Only one of the studies that used the enteric-coated tablets showed positive results: garlic may play an important role in therapy of hypercholesterolemia (Kojuri, Vosoughi et al. 2007).

Quite recently, a parallel-design RCT with raw garlic was performed in the USA (Gardner, Lawson et al. 2007). A number of 192 adults with moderate hypercholesterolemia were randomly assigned to one of the following four treatment groups: raw garlic, powdered garlic supplement, aged garlic extract supplement, or placebo. Garlic product doses equivalent to an average-sized garlic clove were consumed 6d/wk for 6 months. Unfortunately, none of the administered forms of garlic in this study, including raw garlic, had statistically or clinically significant effects on plasma lipid concentrations when given at an approximate dose of a 4-g clove per day for 6 month,. However, an effect might emerge at higher doses, if tolerated.

In 2006 two studies investigating the effect of garlic in cancer patients were published (Ishikawa, Saeki et al. 2006; Tanaka, Haruma et al. 2006). In both studies subjects received aged garlic extract (AGE). Both studies showed positive results. In the study by Tanaka *et al.* (Tanaka, Haruma et al. 2006) the results suggested that AGE suppresses progression of colorectal adenomas in humans. In the study by Ishikawa *et al.* (Ishikawa, Saeki et al. 2006) administering AGE to patients with advanced cancer of the digestive system improved natural-killer cell activity.

Besides garlic, onions are also rich in sulfur-containing allicin compounds. In addition to those compounds, two flavonoid subgroups are found in onions, the anthocyanins, which impart a red/purple color to some varieties, and flavanols such as quercetin and its derivates, responsible for the yellow and brown skins of many other varieties (Griffiths, Trueman et al. 2002).

In conclusion, only a few clinical trials point towards garlic having a role to play in either preventing or delaying cardiovascular disease. Regarding the prevention or delaying of cancer, especially cancer of the digestive system, garlic seems more promising. Therefore, more research concerning the role of garlic in cancer prevention or delaying is recommended. Although there is less information available about onions compared to garlic, onion might be a promising health beneficial product, especially because onion contains other phytochemicals besides sulfur-containing compounds. Therefore, clinical trials with onion are strongly recommended.

2.6 Terpenoids

The group of terpenoids consists of 15.000–20.000 recognized structures. It is distinguished from other classes of secondary metabolites by the common origin from mevalonate and isopentenyl pyrophosphate and by the lipophilic nature of the structures. Terpenes and terpenoids are the primary constituents of many plants and flowers. In nature, terpenoid molecules are implicated in almost every interaction between plant and animal, plant and plant or plant and microorganisms as phytoalexins, insect antifeedants, defense agents, pheromones or signal molecules. Moreover, terpenoids are consumed by humans in their normal daily diet in different mixtures and are therefore responsible for different implications in the human body – a reason why they are discussed as 'functional'.

2.6.1 Monoterpenes

The most investigated monoterpenes are limonene, carvone, carveol and perillyl alcohol but they are only studied in animal models and at cellular level (Wagner and Elmadfa 2003). Based on positive results, human clinical trials investigating the chemotherapeutic potential of monoterpenes are in progress in patients with breast cancer (Crowell 1999; Wagner and Elmadfa 2003). First results show the required doses of monoterpenes with the daily diet which may have clinical activity translates into the consumption of 400 oranges/day!

2.6.2 Tetraterpenes (carotenoids)

Carotenoids are the main group of tetraterpenes and are a group of naturally occurring colorful components that are abundant as pigments in plants. Two main structures in the group of carotenoids are the oxygen-free carotenes like ß-carotene, α-carotene and lycopene, and the oxygenated carotenoids such as lutein, zeaxanthine or ß-cryptoxanthine.
The antioxidative potential, which mainly depends on the number of conjugated double bonds and functional groups, decreases as follow: lycopene > ß-carotene = ß-cryptoxanthine > lutein = zeaxanthine > α-carotene > echinenone > canthaxanthine = astaxanthine (Miller, Sampson et al. 1996).

Beta-carotene

With regard to the well-known fact that ß-carotene is a poor antioxidant at high oxygen pressure, carotenes are not highly effective food antioxidants (Miller, Sampson et al. 1996). However, carotenoids are able to act synergistically, for example with tocopherols, which are very effective at high oxygen pressure thus protecting the organism against free radical reactions.
Epidemiological and intervention studies of the last 10 years have indicated that carotenoids are potential agents for the chemical prevention of carcinogenesis, but in specific groups especially ß-carotene increased cancer mortality. In this field, four main intervention studies have been conducted with predominantly unexpected outcomes. In the Finnish ATBC study (randomized, placebo-controlled) of 29,133 chronic heavy smokers, a significant 16% increase in lung cancer incidence was shown for those who received 20 mg ß-carotene/day over 5–8 years (Heinonen, Huttunen et al. 1994).

Lutein

The association between different carotenoids and colon cancer is especially focused on lutein, which shows an inverse association in both men and women (Slattery, Benson et al. 2000). These data suggest that incorporating lutein-rich food into the daily diet may help to reduce the risk of developing colon cancer. However, long-term intervention studies focusing on the effects of carotenoids on colon cancer risk are still missing.

Lycopene (in tomatoes)

Tomatoes are a valuable source of several micronutrients and phytochemicals including carotenoids, polyphenols, potassium, folate, vitamin C and vitamin E. Dietary intakes of tomatoes and tomato products containing lycopene have been associated with a decreased risk of diseases such as cancer and CVD in numerous studies (Kohlmeier, Kark et al. 1997; Giovannucci, Rimm et al. 2002; Sesso, Liu et al. 2003).

A systematic review of 72 epidemiological studies reported a consistent inverse relationship between intakes of tomatoes and plasma lycopene levels and prostate, lung and stomach cancer (Giovannucci 1999). In the meta-analysis of those studies, 10 out of 14 studies reported a significant inverse relation between tomato or lycopene consumption and lung cancer risk. Epidemiological observations also report an inverse relation between plasma or tissue lycopene levels and the incidence of CVD. Fresh an processed tomato products provide a bioavailable source of lycopene and have a positive correlation with plasma and tissue lycopene levels.

Various mechanisms of lycopene action have been proposed (Hazai, Bikadi et al. 2006), including: (1) antioxidant function (singlet oxygen quenching/radical chain breaking), recently verified in human target tissue as a reduction in 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels after whole-food lycopene intervention; (2) inhibition of cell cycle progression; (3) induction of apoptosis; (4) modulation of gap-junctional intercellular communication (GJIC) and connexin 43 (Cx43) levels; (5) inhibition of insulin like grwothfactor-1 (IGF-1) signal transduction; (6) inhibition of IL-6 expression; (7) induction of Phase II detoxifying enzymes; and (8) inhibition of androgen activation and signaling.

Limited *in vitro* studies with lycopene are performed but these studies do show convincing antioxidant and anticarcinogenic effects of lycopene (Basu and Imrhan 2007). Also animal studies and several clinical trials report beneficial effects following consumption of tomato products containing lycopene. Several studies have shown the antioxidant effects of supplementation of tomato products or purified lycopene (providing 6–17mg lycopene/day), on cellular DNA, in healthy human volunteers (Basu and Imrhan 2007). These studies reveal that the dose and duration of tomato lycopene supplementation, the synergistic action of lycopene with natural carotenoids, the baseline plasma levels of lycopene, the choice of biomarkers of oxidative stress and gene polymorphisms affecting the rate of oxidative stress are critical factors in modulating the response to antioxidant supplementation in healthy volunteers.

Two clinical studies specifically used raw (fresh) tomatoes in the intervention (Visioli, Riso et al. 2003; Riso, Visioli et al. 2004). The Visioli study showed a decrease in LDL oxidiability and urinary F_2-isoprostanes (a marker of *in vivo* oxidative stress) whereas the Riso study showed a decrease in DNA oxidative stress but no effects on lymphocyte MDA levels.

The benefits of tomato intake have been mainly attributed to its lycopene content. It has been shown however that a single compound or class of compounds does not determine the positive effect on health associated with the consumption of fresh fruits and vegetables; rather it is exerted by the whole pool of antioxidants, with noticeable synergistic effects. The importance of synergy of lycopene with other antioxidants in tomatoes is shown in for example the study of Boileau (Boileau, Liao et al. 2003), where male Wistar-Unilever rats showed antineoplastic effects following consumption of tomato powder (13 mg lycopene/kg diet), whereas no effects were observed with lycopene supplementation per se (161 mg lycopene/kg diet). Lycopene synergizes with other natural compounds, such as alfa-tocopherol (vitamin E) and 1,25-dihydroxyvitamin D3, in inhibiting prostate carcinoma cell proliferation (ref Ienucci 2006 *16*), HL-60 leukemic cell differentiation (ref Ienucci 2006 *17*), and LDL oxidation (ref Ienucci 2006 *5*).

In the light of recent clinical trials, a combination of naturally occurring carotenoids, including lycopene, in food sources and supplements, is a better approach to disease prevention and therapy, than administering a single nutrient. Lycopene has shown distinct antioxidant and anticarcinogenic effects at cellular levels, and definitely contributes to the health benefits of consumption of tomato products. However, further research still needs to establish significant health benefits of lycopene supplementation *per se*. The conclusion may be drawn that consumption of naturally occurring

carotenoid-rich fruits and vegetables, particularly processed tomato products containing lycopene, but also fresh tomatoes should be encouraged, with positive implications in health and disease.

2.7 Conclusion

Selection of a phytochemical independent of its matrix has been proven ineffective. In multiple studies it has been shown that a single compound or class of compounds does not determine the positive effect on health; rather it is exerted by the whole pool of antioxidants, with noticeable synergistic effects. Therefore it is essential to take the source and the situation or surrounding wherein a phytochemical originates and develops, the so-called matrix, into account for identifying a fresh fruit or vegetable that is most likely to be approved for certification for a health claim.

Of the phytochemicals studied, the best known and probably most potent flavonoid in relation to health is quercetin. However, supplementation studies with the quercetin compound show almost no effect. This makes the health promoting benefits of quercetin questionable and unfit for fast certification of a health claim. The related kaempferol, and the other group of studied flavonoids, the anthocyans are definitely unlikely to get certified in the near feature since no clinical trails with those compounds were found.

Human intervention studies with proantocyanidins, of the tannin family, show some positive results. The proanthocyanidins were tested however as a part of grape seed extracts. No proanthocyanidin-rich fresh fruits or derived products were tested in these studies, which is essential for the investigation of health effects of fresh foods and thus certification. For an other group of compounds of the tannin family, the ellagitannins, hardly any clinical trials could be found.

A third group of chemicals examined in this study were the vitamins and minerals. Although clinical trails with selenium have been conducted and showed positive results, all the studies found used supplements or selenized yeast and not selenium-enhanced vegetables. Also for the vitamin folate, positive and significant effects were almost always with folate supplements in such a high doses that it is unlikely to be reached by dietary folate.

Of the group of organosulfur compounds, the glucosinolates are the most studied bioactive components, present in the Brassica family. However, human intervention studies are limited in number and scope and so far no positive significant results were found. Another well studied group of organosulfur compounds are the allicins, present in garlic and onion. A large number of clinical trials concerning garlic and its health beneficial effects have been performed. Most of these studies however show no beneficial health effects when garlic extract and/or garlic powder supplementation was used as intervention. Furthermore, only a few clinical trials point towards garlic having a role to play in either preventing or delaying cardiovascular disease.

The last group of phytochemicals are the terpenoids, with the carotenoids as a main group of the tetraterpenes. Both epidemiological and intervention studies of the last 10 years have indicated that carotenoids are potential agents for the chemical prevention of carcinogenesis, but in specific groups especially ß-carotene increased cancer mortality. Several clinical trials however report beneficial effects following consumption of tomato products containing lycopene. Two of those studies specifically used raw (fresh) tomatoes in the intervention model, one of the requirements of PASSCLAIM. These, and other clinical studies have shown the antioxidant effects of supplementation of tomato products or purified lycopene, on cellular DNA and LDL oxidation, in healthy human volunteers. The use of healthy volunteers as a representative of the target group is another PASSCLAIM requirement.

The benefits of tomato intake have been mainly attributed to its lycopene content and lycopene definitely contributes to the health benefits of consumption of tomato products as established by *in vitro*, animal and human studies. Of all the above mentioned compounds, lycopene is the compound which fits the PASSCLAIM criteria the most. The conclusion therefore may be drawn that consumption of naturally occurring carotenoid-rich fruits and vegetables, particularly processed tomato products containing lycopene, but also fresh tomatoes should be encouraged, with positive implications in health and disease. Selection of lycopene in tomatoes as the most promising phytochemical and matrix that is most likely to be approved for certification for a health claim within limited time therefore seems a logical choice.

3 Tomatoes (lycopene) as a fresh functional food

As shown in the previous section of this paper, experimental and clinical studies have demonstrated that lycopene exhibits antioxidant activities, suppresses cell proliferation and interferes with the growth of cancer cells. Also in epidemiologic studies, the beneficial effects of tomatoes have been demonstrated. For example, tomatoes are a main component of the traditional Mediterranean diet, which has been found to be associated with a low rate of mortality due to cardio-vascular disease.
In this second section of the report, factors potentially important for certification of tomato as a fresh functional food product will be discussed. At first, the literature about lycopene bioavailability in humans will be discussed. Then, the literature about possible mechanisms behind the health effects of lycopene will be evaluated. Next, potential adverse and synergetic effects of lycopene intake will be addressed. In relation to the substantiation of a health claim the following aspects are discussed; the potential target group, relevant biomarkers and dose-response relation. The antioxidant content of tomatoes depend on genetic and environmental factors, agricultural techniques, post-harvest storage and processing conditions and will be discussed in the last part of this report.

3.1 Characteristics of lycopene

3.1.1 Lycopene

Lycopene is an acyclic carotenoid which contains 11 conjugated double bonds arranged linearly in the all-*trans* form (Stahl and Sies 1996). In most foods, lycopene and other carotenoids typically occur in this configuration, which is thermodynamically the most stable form (Clinton 1998). Light, heat or chemical reactions may transform all-*trans* lycopene into various *cis*-isomers (see figure 4) (Shi, Qu et al. 2004).

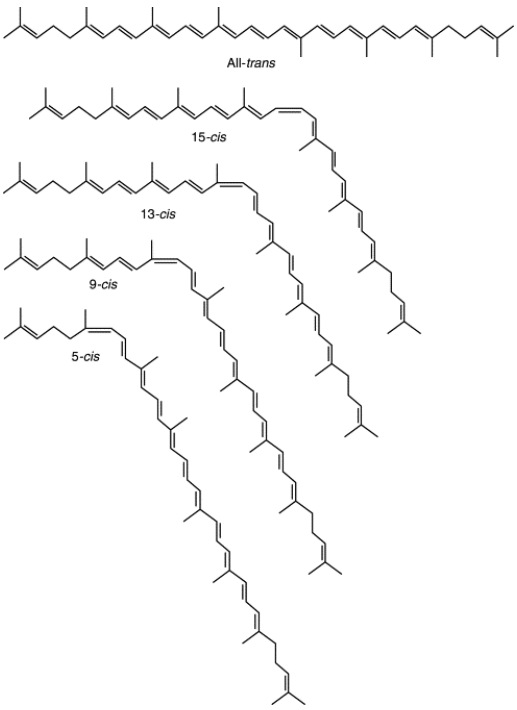

Figure 4. Structures of *trans* and *cis*-isomeric forms of lycopene (Rao, Ray et al. 2006)

3.2 The pharmacokinetics of lycopene

3.2.1 Absorption

Carotenoids appear to be absorbed by duodenal mucosal cells by passive diffusion, just like cholesterol and the products derived from triglyceride lipolysis (Parker 1996). In the small intestine lycopene is incorporated into micelles, which facilitates the absorption into the intestinal mucosa cells via passive transport (Stahl and Sies 1996).
Micellar inclusion capacity of carotenoids may depend on their structural features and micellar lipid composition (Parker 1996; Clinton 1998). Incorporation into micelles may be limiting in human absorption in case of high carotenoid intake (> 20 mg). With lower (< 10 mg) amounts, the limiting factor is more likely the release from food particles and the dissolution in bulk lipid (Parker 1996).
The efficiency of carotenoid release from the food matrix depends on the physical disposition of carotenoids in the matrix, the particle size after mastication and stomach action, and the efficiency of digestive enzymes (Parker 1996).
In plants, carotenoids are associated with proteins, which reduces their bioavailability. These protein-carotenoid complexes are weakened by heating (Parker 1996; Clinton 1998). Also dissolution or dispersion of crystalline carotenoid complexes may be induced by heating, which has clearly been demonstrated for lycopene from tomato products (Parker 1996).
Since the uptake of lycopene is probably due to passive diffusion, the rate of diffusion depends on the concentration gradient between the micelle and the plasma membrane of the enterocyte. Since the water solubility of carotenoids is very low, contact between the micelle and the cell membrane is probably necessary. Factors that impair movement of micelles or their contact with the mucosa are likely to impair carotenoid uptake. Such a factor is, for example, dietary soluble fibre and non-digestible fat, which may act like a hydrophobic sink (Parker 1996). Dietary interactions are further discussed below.
Goni *et al.* have investigated the bioaccessibility of major dietary carotenoids using an *in vitro* gastrointenstinal model. Small intestine bioaccessibility of lycopene in carotene-rich fruits and vegetables (including tomato) differed considerably (4 and 44%, respectively). Whether bioaccessible lycopene is really absorbed, depends among others on incorporation of lycopene into micelles and the interference of the soluble indigestible fraction of the food matrix with micelle formation (Goni, Serrano et al. 2006). Bioaccessibility of carotenoids in the large intestinal model was determined after fermentation of the indigestible fraction from the small intestine by rat derived colonic microflora. In the fruit group over 70% of the lycopene present was released during this process and circa 15 % remained unavailable. Lycopene release in the vegetable group was 54% and only 2% remained unavailable (Goni, Serrano et al. 2006). Whether lycopene is indeed absorbed in the large intestine needs to be investigated. Despite its apparent high bioaccessibility, only 10-30% of the consumed lycopene is absorbed in humans, the remainder of the lycopene is excreted (Shi, Qu et al. 2004). If carotenoids are not repackaged into chylomicrons within 3 days after uptake by the mucosa, enterocytes are sloughed off into the lumen of the GI-tract and their carotenoid content is lost (Williams, Boileau et al. 1998).
The intestinal translocation and metabolism of lycopene is still unknown (Parker 1996; Stahl and Sies 1996).

3.2.2 Distribution

Lycopene in the circulation

Carotenoids are exclusively transported in lipoproteins in plasma (Parker 1996).
Intact carotenoids are incorporated into chylomicrons which are secreted into the bloodstream via the lymphatic system (Parker 1996; Stahl and Sies 1996).

In blood plasma carotenoids are initially in the chylomicron and VLDL fraction, whereas the levels of carotenoids associated with other lipoproteins such as LDL and HDL rise at later time points with peak levels at 24-48 hours (Stahl and Sies 1996). However, peak levels of lycopene after already 5 hours are also found (Johnson, Qin et al. 1997).
The distribution of lycopene within the lipoprotein fractions is similar to that of β-carotene, α-carotene and cholesterol: LDL: 58-73%, HDL: 10-16%, and VLDL: 10-16% (Parker 1996). Hence, the major vehicle of lycopene is LDL, whereas other more polar carotenoids are more equally distributed between LDL and HDL (Stahl and Sies 1996). Distribution of carotenoids within lipoproteins depends on physical properties of the carotenoid. Lycopene, because of its hydrocarbon structure, resides exclusively in the hydrophobic core of the lipoprotein. This may have an effect on the transfer to other lipoproteins during circulation, or their uptake by extrahepatic tissues (Parker 1996).
β-Carotene may be transferred from chylomicron to HDL in the circulation during lipolysis. Only a small portion of β-carotene is transported by HDL. Apparently there is limited carrying capacity for hydrocarbon carotenoids. The amount of β-carotene transferred to HDL is probably small compared to the amount retained by the chylomicron remnant and internalized by the liver for secretion in VLDL (Parker 1996). Whether this process also holds for lycopene is not known.
Mean serum concentration of lycopene in different populations range from 50 – 900 nM, although interindividual variation is very large (Clinton 1998). Lycopene is found to contribute between 21 and 43% of total carotenoids in human serum (Stahl and Sies 1996).
An intervention study by Allen *et al.* showed a 50% decrease in blood lycopene after a 2-week lycopene-free diet. When participants consumed a single, daily standard serving of tomato products, blood concentration appears to reach a plateau after approximately 2 weeks (Allen, Schwartz et al. 2003).
Clinton states that in general total serum lycopene changes gradually with alterations in dietary intake and that chylomicron intake is a better approach for assessing recent meals (Clinton 1998). In general a chylomicron peak occurs at 4-6 h and declines to near-baseline concentrations within 12 h. In addition, carotenoid concentration in chylomicrons mainly reflects absorption kinetics (Gartner, Stahl et al. 1997).
In a study of Gärtner *et al.* chylomicron response was significantly higher after consumption of tomato paste compared to fresh tomatoes as assessed by chylomicron lycopene concentrations, area-under-the-curve responses and peak concentrations. The peak lycopene concentration in chylomicrons occurred in 4-6 h post dose. In serum no distinct changes in lycopene concentrations were observed (Gartner, Stahl et al. 1997).
Through the action of lipoprotein lipases on chylomicrons, lycopene has the potential to be taken up passively by various tissues, including adrenals, kidney, adipose tissue, spleen, lung and reproductive organs before clearance of chylomicron remnants in the liver via the chylomicron receptor. Carotenoids can accumulate in the liver or can be re-packaged into VLDL and sent back into the blood (Boileau, Boileau et al. 2002).

Lycopene in tissues

Lycopene is the predominant carotenoid in the human liver, adrenal glands, adipose tissue, testes and prostate (Boileau, 2002 #6}. The processes that underlie these patterns remain speculative (Clinton 1998). Moreover, between-person variation for tissue concentrations of lycopene is very high (Clinton 1998). Though, it is suggested that the tissue concentrations of carotenoids may be linked to the relative number of LDL receptors or relative uptake of lipoproteins among tissues. In addition, tissue concentration may reflect differences in their relative metabolic or oxidation rates (Erdman 2005).
The level of carotenoids in adipose tissue is relatively low in comparison to, for example, liver or testes, though lycopene is very lipophilic. However, the amounts of lycopene in adipose tissue contribute considerably to the total content in the organism, since adipose tissue represent about 20% of total body weight (Stahl and Sies 1996). Adipose tissue carotenoid levels are lower in men (25-50%) as compared to women. Body mass index and waist circumference were inversely related to adipose tissue lycopene content (Clinton 1998).

In contrast to blood levels, the lycopene content of buccal mucosal cells was not significantly changed after a 2-week lycopene-free diet, though levels were elevated after 2 weeks with daily consumption of tomato sauce (Allen, Schwartz et al. 2003).
Standard sized portions of tomato products were found to increase plasma and breast-milk lycopene concentrations in lactating women and therefore could increase the lycopene status of nursing infants (Schwartz 2005). The concentrations of lycopene in human milk are circa 10% of those in serum (Clinton 1998). Further research is necessary to determine the relationship among the intake of lycopene, concentrations in human milk and transfer to the infant (Clinton 1998).
The lycopene levels of immunoinfertile men were significantly decreased in seminal plasma compared to those of fertile men, which may suggest a role for lycopene in male reproduction and fertility (Clinton 1998). However, a study with castrated rats showed a doubling of hepatic lycopene, despite a lower lycopene consumption. In addition, food restriction caused a testosterone reversible increases of hepatic lycopene. Based on this study Erdman states that with higher androgen levels or greater consumption of energy , there may be enhanced lycopene metabolism and degradation (Erdman 2005).

Metabolism and excretion

Little is known about metabolism and degradation of lycopene. Stahl *et al.* reported that intact carotenoids are excreted with bile (Stahl and Sies 1996). Several oxidation products of lycopene have been identified in human serum (Williams, Boileau et al. 1998). The major metabolite of lycopene identified in human plasma is 5,6-dihydroxy-5,6-dihydrolycopene, probably due to the oxidation of lycopene via conversion from intermediate lycopene epoxides (Shi, Qu et al. 2004). However, the exact mechanisms of formation and the site of formation of these metabolites are unknown (Williams, Boileau et al. 1998).
Elimination of lycopene from plasma after ingestion of processed tomato juice occurred with a half-life of about 2-3 days (Stahl and Sies 1996). However, a half-life of 7-14 days of lycopene in human blood is more often reported (Shi, Qu et al. 2004).

All-*trans*-lycopene versus its *cis*-isomers

Though circa 90% of dietary lycopene is found to be in the all-*trans*-conformation, *cis*-lycopene is found to be the main lycopene present in human tissues (Boileau, Boileau et al. 2002). The most prominent lycopene isomers in human plasma are all-*trans*-lycopene and 5-cis-lycopene (Boileau, Boileau et al. 2002; Allen, Schwartz et al. 2003). There is however very little known about specific roles of *cis* or *trans*-isomers in vertebrate biology (Clinton 1998). The *cis*-isomer formation *in vivo*, the metabolism and introversion of isomers, and biological effects or functions of specific isomers are all poorly understood (Allen, Schwartz et al. 2003).
Thermal treatment and processing result in only small increases (<10%) in *cis*-lycopene content of foods. Also exposure to the low pH of the stomach results only in a small increase in *cis*-lycopene isomers. This indicates that other mechanisms are responsible for the large all-*trans* : *cis*-isomer ratios observed between foods and tissues (Boileau, Boileau et al. 2002).
To test the hypothesis that *cis*-isomers of lycopene are more bioavailable, Boileau *et al.* prepared bile acid micelles containing crystalline lycopene (54,4% *cis*). The *in vitro* micelle preparation contained 75.9% *cis*-lycopene. This observation may be explained by the fact that the *cis*-conformation shortens the effective chain length compared to the all-*trans* form. *Cis*-isomers are also less likely to aggregate than the all-*trans* isomer, and therefore can be more easily incorporated into micelles (Boileau, Merchen et al. 1999). In the same study ferrets (a good model for carotenoid absorption) were used to study the *in vivo* absorption of lycopene. The lycopene fed to the ferrets contained 90% all-*trans* isomers, comparable to the isomer pattern of tomato (products). The isomer profile of the stomach and intestinal content did not differ significantly from the original dose. However, the mucosa contained a significantly greater proportion of *cis*-isomers (58.8%). The lycopene profile in lymph secretion (77.4% *cis*) was significantly larger than all other tissues (Boileau, Merchen et al. 1999). At high dose absorption is dependent on two concentration gradients: micelle to brush border membrane

and from the membrane to an intracellular location. *Cis*-lycopene may be faster incorporated into chylomicrons, which could be an explanation for the higher concentration of *cis*-isomers in lymph than in mucosa (Boileau, Merchen et al. 1999).
While over 75 % of lycopene in the lymph is in the *cis*-conformation, human plasma consists of an isomer mixture with the *cis*-isomers contributing about 50% to total lycopene (Stahl and Sies 1996; Boileau, Merchen et al. 1999). Lung and liver tissue have a comparable distribution. A similar isomer profile is observed when lycopene is stored in organic solvents. This mixture is probably most stable and represents an equilibrium between trans lycopene and its isomers (Boileau, Merchen et al. 1999).
Another study, however, reported an all-*trans* : *cis*-isomer ratio of 40-60 in human subjects at baseline. After a 2-week lycopene-free diet this ratio significantly changed to 30:70. After a 2-week intervention with tomato products, the ratio had returned to baseline levels (Allen, Schwartz et al. 2003). Similar *trans:cis* ratios (40-60; 30-70; 40-60) have recently been reported (Frohlich, Kaufmann et al. 2006). The observed decrease of all-*trans*-lycopene may be due to a more rapid clearance, greater tissue uptake and conversion to *cis*-isomers of all-*trans*-isomers. Also the possible mobilization of lycopene stores from tissues, where lycopene is predominantly found in the *cis*-from, may contribute to a relative increase in the plasma *cis*-isomer pool (Allen, Schwartz et al. 2003). All-*trans* : *cis*-isomer ratio in buccal mucosal cells did not significantly change during both the lycopene-free diet and intervention phase (Allen, Schwartz et al. 2003). Also in a pilot clinical trial, the prostate all-*trans*: *cis*-isomer ratio in prostate cancer patients did not differ between the subjects consuming tomato-based products and subjects consuming soy after the treatment phase (Schwartz 2005).
Gärtner *et al.* reported that all-*trans*-lycopene accounted predominantly (65%) for the total lycopene response in chylomicrons. It was suggested that that lycopene appears to be absorbed into chylomicrons mainly in the form that is present in foods and is isomerized *in vivo* to yield the typical pattern found in serum and other tissues (Gartner, Stahl et al. 1997). These findings are however not in line with the previously mentioned results of Boileau *et al.*, which suggested a higher bioavailability of *cis*-isomers.
An *in vitro* study with castrated rats showed increased accumulation of total lycopene and *cis*-lycopene while eating less total lycopene than normal rats. It was also found that the percentage of lycopene in the *cis*-conformation increased with increased concentrations of dietary lycopene. Therefore hormonal and physiological factors may play a role in regulations of *trans-cis* ratio (Boileau, Boileau et al. 2002).

3.2.3 Factors influencing bioavailability

Possible interaction with other food compounds

Absorption of lycopene may be influenced by dietary lipid, certain fibers, plant sterols, presence of vitamins, minerals and other carotenoids (Boileau, Boileau et al. 2002; Shi, Qu et al. 2004). Bile production is stimulated by dietary fat, which increases the efficiency of the absorption of lycopene by the formation of micelles (Boileau, Boileau et al. 2002). The effects of fats and fibers on lycopene bioavailability are extensively reviewed in paragraph 3.8.
After combined oral administration of β-carotene and lycopene in humans, the absorption of lycopene was significantly increased (compared to lycopene alone). β-Carotene absorption was not affected (Johnson, Qin et al. 1997). However, contradictory effects have also been reported (Parker 1996; Shi, Qu et al. 2004).
Co-ingestion of lycopene with lutein diminished lycopene content in chylomicron fraction and *vice versa*. In plasma however, lycopene was significantly increased after simultaneous intake of tomato purée with lutein-pills as compared to tomato purée alone (Shi, Qu et al. 2004). Lycopene and canthaxanthin are reported to exhibit antagonism for absorption and distribution to the liver (Clinton 1998). No effect of lycopene on plasma concentrations of zeaxanthin, α-carotene, and β-cryptoxanthin were found (Shi, Qu et al. 2004). However, competition between lycopene and other carotenoids has to be further elucidated.
Intraduodenal infusion of soybean phosphatidylcholine significantly increased lymph lycopene levels in rats, especially in combination with α-tocopherol (Nishimukai and Hara 2004).

Levels of ascorbic acid (vitamin C) and the fat soluble tocopherols (vitamin E) were affected neither by depletion, nor by supplementation of lycopene (Frohlich, Kaufmann et al. 2006).

Other factors influencing bioavailability

There are many factors associated with a change in lycopene levels. Increasing age is correlated with gradual incline in serum concentrations. In addition lipid malabsorption, caused by either disease or drugs, and lower non-HDL cholesterol may inhibit lycopene uptake (Stahl and Sies 1996; Clinton 1998). Also cholesterol lowering drugs that interfere with the incorporation of lycopene into micelles can potentially decrease the efficiency by which carotenoids are absorbed (Boileau, Boileau et al. 2002).
Most studies have not found a significant effect of gender on lycopene absorption. However, a lycopene peak is observed middle in the luteal phase of the menstrual cycle. This can influence estimation of lycopene-disease relationships in studies of pre-menopausal women and should therefore be taken into account (Clinton 1998).
Alcohol consumption with associated liver dysfunction, both sever and modest, show significantly reduced levels of hepatic lycopene. Serum concentrations demonstrate less consistent alterations in lycopene levels (Clinton 1998). Also the effect of smoking on lycopene levels is equivocal (Clinton 1998).
Hypothyroidism, anorexia nervosa, bulimia and weight loss are associated with elevated plasma carotenoids, irrespective of diet (Erdman 2005).

The studies mentioned above clearly show that lycopene is bioavailable. The absorption process of lycopene in the GI-tract is well understood compared to other kinetic factors. However, the effects of the food matrix on lycopene absorption and the fate of all-*trans*-lycopene and its *cis*-isomers in the gut are subjects for further research. Matrix effects and other events in the upper GI-tract are however difficult to study in humans. Therefore, the use of *in vitro* digestion models and for example *in vivo* ferret models is suggested to gain further insight on this topic.
Long term lycopene status, in both blood and tissues, needs to be further explored to investigate the effects of a diet rich in tomatoes. Also tissue specific biological actions are of interest.
Hardly anything is known about metabolism and excretion of lycopene. More research in this field may yield new metabolites of lycopene and provide information on possible mechanisms. Concerning the *cis*-isomers and all-*trans*-confirmation of lycopene, the possible *cis*-isomer formation *in vivo*, the metabolism and introversion of isomers, and biological effects or functions of specific isomers are of interest for further investigation.

3.3 Mechanisms of lycopene on health

Evidence for a health promoting effect of lycopene in humans is primarily derived from human intervention studies. However, the mechanism behind this effect is not yet clearly understood. Mechanistic studies in animals and to a lower extent cell line studies contribute to the strength of the evidence. In this chapter some possible mechanisms will be explained.

The health beneficial effects of lycopene can be divided into antioxidant activity and non-antioxidant activity. Antioxidant activity is a preventive mechanism involved in reducing oxidative stress, thus preventing or delaying the occurrence of chronic diseases like cardiovascular diseases, cancer, and probably osteoporosis. Non-oxidative activity consists of mechanisms involved in repair of the damage induced by reactive oxygen species (ROS) or carcinogenic compounds. A schematic overview of prevention and repair mechanisms is shown in figure 5. These repair mechanisms include gene function regulation and intercellular gap junction communication. Furthermore, lycopene acts as a hormone- and immunomodulator, it inhibits signal transduction pathways involved in carcinogen metabolism and it enhances detoxifying phase II enzymes like glutathione peroxidase (Liu 2004; Rao and Ali 2007).

Prevention mechanisms

– Antioxidant activity

Repair mechanisms

– Gene function regulation

– Improving gap junction communication

– Hormone- and immunomodulation

– Decreasing carcinogen metabolism

– Enhancing phase II enzymes.

Figure 5. Mechanisms through which lycopene is involveld in the inhibition of chronic diseases (Rao and Ali 2007).

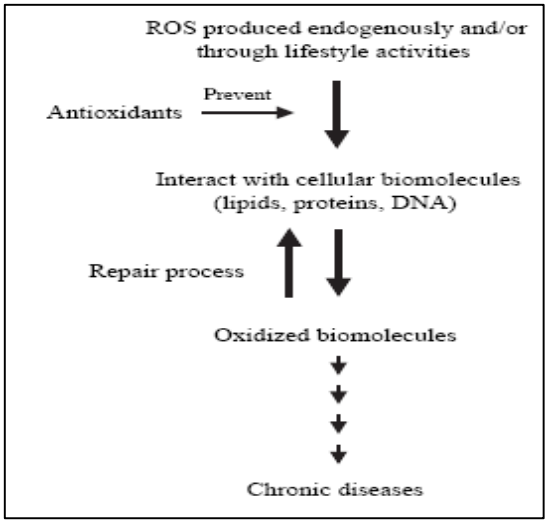

Figure 6. Lycopene interference in chronic disease development (Rao and Ali 2007).

3.3.1 Prevention mechanisms

According to many clinical trials concerning the antioxidative activities of lycopene (Appendix IX), lycopene protects LDL from oxidation. This protective effect is contributed to the antioxidative properties of lycopene. A positive association is known between oxidized LDL (LDLox) and atherosclerosis (Stocker and Keaney 2004), the major risk factor for stroke and myocardial infarction (Lorenz, Markus et al. 2007). The mechanism involved in preventing LDL from oxidation by ROS is difficult to consider. It is Important that lycopene is transported in the blood in the highly lipophilic core of LDL. Incorporation of lycopene in LDL probably prevents LDL from oxidation of the apoprotein B100 (apo-B100) protein by direct antioxidative activity (Stocker and Keaney 2004).

Besides the antioxidative properties of lycopene on LDL oxidation which is possibly associated with CVD, lycopene seems to be involved in the prevention of oxidative DNA-damage, which in turn is

associated with several cancers. Progression of a tumour consists of three stages called initiation, promotion and progression. The first step is caused by oxidation of DNA or chromosomes by carcinogens like ROS. Antioxidants are thought of to play a preventive role in this process by decreasing DNA-damage. Data from clinical trials support this antioxidative protection on DNA-damage (Basu and Imrhan 2007) (Appendix IX). The anticarcinogenic effect of lycopene is different for certain types of cancer. Epidemiological studies show an inverse association between serum and tissue lycopene and the risk of prostate caner, breast, cervical and ovarian cancers, cancers of the gastrointestinal tract and lung cancers (Giovannucci 1999; Rao, Ray et al. 2006). According to a study on tissue distribution of lycopene, a weak relationship was seen between tissue distribution and cancer risk (Rao and Agarwal 1999). Lycopene is known to accumulate in tissues, and the concentration of lycopene differs per organ. The protective effect of lycopene on prostate cancer may for example be due to the high concentration of *cis*-lycopene (approximately 80 % of all lycopene) (Clinton, Emenhiser et al. 1996; Boileau, Boileau et al. 2002). The concentration differences between organs are possibly caused by large amounts of LDL-receptors in the mentioned organs; it is presumed that lycopene is transported by LDL in the blood (Rao and Agarwal 1999). Another reason can be to *in vivo* isomerisation of all-*trans*-lycopene into *cis*-lycopene. There are some indications, but no known mechanism can support this theory (Rao, Ray et al. 2006).
The role of lycopene in preventing DNA-damage may be ascribed to the direct antioxidant activity. According to a study by Chasse *et al.,* in tissues containing a small ratio of all-*trans*/*cis*, antioxidant activity is higher, for example in prostate tissue (Chasse, Mak et al. 2001).
As shown in figure 6, preventive mechanisms only decrease the oxidation rate of lipids, proteins and DNA. Oxidized biomolecules can result in the development of certain chronic diseases, in this step lycopene is involved in repair and breakdown of damaged molecules.

3.3.2 Repair mechanisms

Gene function regulation

Lycopene has inhibitory effects on cell growth by regulation of genes involved in cancer development. The modes of action are not well known, but some indications have been found.
Lycopene seems to downregulate cyclin D1 (and cyclin D3), proteins involved in cell proliferation. This inhibition leads to cell cycle arrest in prostate cell lines, promyelocytic leukaemia cells, breast cancer cells and endothelial cancer cells (Wertz, Siler et al. 2004). Besides prostate cancer cells, also normal prostate cells are sensitive to the inhibition effect of lycopene. This is beneficial to the prostate, because benign prostate hyperplasia is positively associated to prostate cancer.
Few studies have been performed on induction of apoptosis by lycopene. These studies suggest that some oxidative metabolites of lycopene induce apoptosis, but lycopene concentrations in these studies are outside the physiological range (Kotake-Nara, Kim et al. 2002) (Nara, Hayashi et al. 2001). However, a clinical trial showed a significant increase in apoptotic index of patients with prostate cancer, in prostate cancer cells and hyperplastic cells (Bowen, Chen et al. 2002). However, if lycopene contributes to the induction of apoptotic processes in cancer cells, the mechanism for this effect is still unknown.

Gap junction communication

Healthy cells communicate with surrounding cells by connecting canals consisting of proteins synthesized through transcription of the connexion 43 gene. Gap junction communication contributes to tissue homeostasis and is involved in the exchange of small molecules like nutrients and signalling molecules (Tapiero, Townsend et al. 2004). Cancer cells have shown to have a decreased gap junction communication compared to normal cells. Thus, enhancement of connexin proteins may be a promising therapeutic method to inhibit cancer growth (Parmender P. Mehta 1999).
Induction of intercellular communication by gap junctions can be achieved by lycopene, and is associated with growth inhibition in cancer cells (Tapiero, Townsend et al. 2004). Lycopene seems to enhance transcription of the connexion 43 gene, thereby increasing the amount of gap junctions.

Hormone- and immunomodulation

Steroid hormones like androgens seem to promote prostate cancer when exposed over a period of time. Cell growth is increased by conversion of testosterone into 5-α-dihydrosterone (DHT) in both normal prostate cells as well as in prostate cancer cells. DHT interacts with the androgen receptor AR, leading to activation of several genes involved in cell growth. Lycopene has shown to downregulate 5-α-reductase I in cancer cells, responsible for the conversion of testosterone into DHT (Wertz, Siler et al. 2004).
Few studies are performed on immunomodulation by lycopene. A study on T-cell differentiation in mice indicates that lycopene normalizes T-cell differentiation, caused by tumorigenesis (Kobayashi, Iijima et al. 1996). Differentiation of T-cells is thought to have a protective effect on cancer.

Decreasing carcinogen metabolism

An important mechanism contributing to the protective effects of lycopene against cancer seems to be the inhibition of IGF-I. IGF-I shows a causal relationship with several cancers, including prostate cancer. This has been shown in transgenic adenocarcinoma of the mouse prostate (TRAMP) cancer model and in healthy transgenic mice developing prostate cancer after overexpression of IGF-I in prostate epithelial cells (Wertz, Siler et al. 2004).
The possible mechanism is binding of lycopene to IGF-I-binding protein, thereby inhibiting IGF-I signal pathways (Karas, Amir et al. 2000). In addition to these animal studies and cell line studies, serum IGF-I concentration was assessed in clinical intervention studies. Data are controversial, some studies show a decrease in IGF-I, while other studies do not support this effect. This controversy is possibly due to the fact that a large part of IGF-I is produced in the liver and a decrease in IGF-I production in the prostate can not easily be detected in the serum (Wertz, Siler et al. 2004).
Another pathway in which lycopene is involved is the interleukin-6 pathway. Interleukin-6 (IL-6) promotes inflammation and acts as a paracrine and autocrine growth factor in prostate epithelial cells (Wertz, Siler et al. 2004). Prostatitis caused by IL-6 is associated with increased cell turnover, and therefore may be seen as a risk factor for cancer (De Marzo, Meeker et al. 2003). Moreover, IL-6 is an activator of the androgen receptor, thereby increasing the transcription of carcinogenic proteins by binding of DHT to the androgen receptor (Culig, Bartsch et al. 2002).
Lycopene possibly decreases the expression of IL-6 in the MatLyLu rat prostate tumor model. This effect may contribute to the protective effect of lycopene on prostate cancer (Wertz, Siler et al. 2004).

Enhancement of phase II enzymes

Phase II enzymes act as indirect antioxidant, and play an important role as first barrier to small molecular agents, including exogenous carcinogenics (Wertz, Siler et al. 2004). Prostate cancer tissue has diminished antioxidative activity of phase II enzymes. Prostate cancer tissue as well as inflammated prostate tissue have an increased amount of silenced glutathione-S-transferase π1 (a detoxifying phase II enzyme), due to methylation of the involved gene (Lee, Isaacs et al. 1997).
Moreover, lycopene enhances the activity of several phase II enzymes, including glutathione-S-transferase (GST), glutathione peroxidase (GP), glutathione reductase (GR) and glutathione (GSH). The mechanism of increase is not fully understood, but may be due to the antioxidant activity of lycopene, mediated by the antioxidant responsive element in promoter regions of abovementioned phase II enzymes (Dhakshinamoorthy, Long 2nd et al. 2000).

In conclusion, several mechanisms are more or less known. In general, lycopene is involved in various of mechanisms, either by direct antioxidative activity or by enhancing or inhibiting pathways involved in carcinogenesis. Difficulties arise if one of these mechanisms is regarded as the protective mechanism on its own. More reasonable is that several mechanisms together cause the protective effect on health. Moreover, these mechanisms account for the protective effects on several cancers, but the effect on prostate cancer is by far the most studied. A difficulty is that results are obtained from animal studies and studies in cell lines. Data from clinical trials are difficult to obtain. Moreover, results

are derived from many studies using lycopene supplementation, instead of administering tomato products.
An important gap in the knowledge about beneficial effects of lycopene on health is whether the effects are caused by lycopene itself, or by one of its metabolites. Lindshield *et al.* believe that lycopene metabolites largely contribute to the beneficial effects on health (Lindshield, Canene-Adams et al. 2007). However, research in this direction is limited and more research is needed.

3.4 Synergy between lycopene and other tomato phytochemicals

In the previous chapter, several mechanisms are explained which could inhibit oxidative stress and cancer growth. Mechanistic studies are primarily conducted in cell lines. Treatment in these studies consists primarily of lycopene supplements. Focus in clinical trials is on tomato products, either raw or as processed tomato products. Thus, consideration of a synergetic effect of lycopene with other phytochemicals present in tomatoes, is important.

A study performed by Boileau *et al.* focused on the difference in survival between rats induced with prostate cancer after intervention with beadlets containing tomato powder, lycopene or a placebo. Survival was significantly higher in the group receiving tomato powder compared to lycopene (Boileau, Liao et al. 2003). This suggests there is a form of synergy between lycopene and one or more phytochemicals in the tomato.

Phytochemicals present in tomatoes are besides lycopene, vitamins C and E (α-tocopherol), carotenoids β-carotene, lutein, phytoene, phytofluene and γ-carotene, and some flavonoids, like quercetin and kaempferol. Studies on synergy between lycopene and other phytochemicals are primarily *in vitro*.

A study performed in a mouse model for human prostate cancer showed an inhibition on plasma Prostate Specific Antigen (PSA) levels of lycopene in combination with α-tocopherol, while lycopene and α-tocopherol alone showed no inhibition (Limpens, Schroder et al. 2006).

A study conducted in human LDL *in vitro* compared the effects of lycopene, β-carotene, α-tocopherol alone and the synergy between lycopene and β-carotene and α-tocopherol, respectively (Fuhrman B 2000). This study shows a significant two-fold increase by synergetic effect between lycopene and α-tocopherol on LDL oxidation in the biomarkers TBARS and lipid peroxides.

The study by Stahl *et al.* measured the decrease in TBARS formation from lipid peroxidation by carotenoids and α-tocopherol alone and the contributing synergetic effects. They showed that a carotenoid mixture containing the major carotenoids found in human plasma and tissue, together with α-tocopherol had lower amounts of TBARS, thus lower oxidation (Stahl, Junghans et al. 1998).

The possible mechanism of synergy between lycopene and α-tocopherol is that lycopene is situated in the inner core of LDL, while α-tocopherol is situated at or near the membrane. Lycopene and α-tocopherol are thought of to protect LDL from oxidation at specific sites. In addition, lycopene and α-tocopherol seem to protect each other from oxidation, thereby reaching the synergetic effect (Shixian, Da et al. 2005).

Pastori *et al.* assessed the effect of lycopene, α-tocopherol and the synergetic effects of lycopene and α-tocopherol on proliferation in prostate cancer cell lines (DU-145 and PC-3). In contrast to previous study results, lycopene alone did not inhibit proliferation, α-tocopherol did, but the greatest inhibition of proliferation was seen when lycopene and α-tocopherol worked synergetically together (Pastori, Pfander et al. 1998). The authors concluded that the synergetic effect was not due to antioxidant activity, but primarily to their different effects on signal transduction in prostate cancer cells.

In conclusion, as supported by data from animal studies, intervention of tomato products shows higher response in oxidative stress and carcinogenic processes, compared to intervention of lycopene alone. This suggests synergy between lycopene and for instance α-tocopherol. Data from cell line studies shown in this chapter support this theory, but the mechanism is still unclear. Furthermore, the optimal ratio between lycopene and α-tocopherol in the diet and blood plasma is unknown. Also, possible synergy between lycopene and vitamin C and flavonols like quercetin and kaempferol should be investigated.

3.5 Assessment of the adverse effects of lycopene

Many clinical trials have been performed on the health promoting effect of lycopene in the form of tomatoes, tomato products or as synthetic lycopene. Assessment of the possible adverse effects of excessive, prolonged intake of lycopene should also be undertaken.

The review of Shao and Hathcock (Shao and Hathcock 2006) assessed the presence of adverse effects by determining the upper level of supplementation (ULS), observed safe level (OSL) and the highest observed intake (HOI), all determined by the US Food and Nutrition Board and adopted by the Food and Agricultural Organization (FAO) of the WHO. Data from clinical trials lead to a ULS of 75 mg/day and an OSL of 75 mg/day. These data are obtained from *trans*-lycopene or total lycopene doses used in clinical trials. A No observed adverse effect level (NOAEL) can not be identified, because no adverse effects were reported from any clinical trials. The highest dose administered was 150 mg/day, thus the NOAEL is >150 mg/day (Shao and Hathcock 2006).

Based on animal studies, the highest acute dose where no adverse effects were seen was 5000 mg/kg body weight and 4500 mg/kg body weight for chronic and sub-chronic toxicity. This NOAEL detected in animals can be extrapolated to humans, using an uncertainty factor of 1000, leading to a ULS of 270 mg/day (Shao and Hathcock 2006).

Compared to a mean lycopene intake in the United States of 8 mg/day, the intake of lycopene as supplement can regarded as safe. The use of lycopene from fresh tomatoes or tomato products can be regarded as safe, due to the low bioavailability compared to lycopene bioavailability from supplements. In addition to the data mentioned above, the Food and Drug Administration in the US granted nutritional supplementation of lycopene as generally recognized as safe (GRAS) (Rao, Ray et al. 2006).

The only adverse effect seen in humans was reported in a case study by Reich *et al.* in 1960. A 61 years old woman showed slight reddishness of the skin because of lycopenemia (accumulation of lycopene in the skin) after consumption of two liters of tomato juice daily for several years. After a 3-week free tomato diet, the color faded. (Reich, Shwachman et al. 1960).

3.6 Substantiation of a health claim for tomatoes rich in lycopene

Various studies have demonstrated the function of tomatoes on human health. As mentioned in the previous parts of this report, lycopene is the assumed health beneficial phytochemical in tomatoes. As illustrated in figure 7, the effects of tomatoes on human health are diverse and concern a range of diseases, from several types of cancer to neurodegenerative diseases (Rao, Ray et al. 2006). Increased oxidative stress has been implicated in the incidence of chronic diseases, such as cancer and cardiovascular disease (Basu and Imrhan 2007). Dietary intake of tomatoes and tomato products containing lycopene have been mainly associated with oxidative stress (Basu and Imrhan 2007). Although, many mechanistic studies have investigated the role of antioxidative as well as non-antioxidative activity of lycopene, antioxidative activities are by far more studied in clinical trials. Therefore, a claim on the target function oxidative stress, rather than on a disease, is the most reasonable for the near future. On somewhat longer term, a claim on the reduction of a disease risk may be aimed for. As cancer, prostate cancer in particular, in relation to tomatoes/lycopene has been studied the most (Rao, Ray et al. 2006), it is considered as the most promising for a reduction-of-disease-risk claim. Nevertheless, this chapter will focus on the substantiation of the role of tomatoes and tomato products on reduction of oxidative stress in humans. In particular, the target group for the claim, the use of biomarkers to proof the efficacy of tomatoes/lycopene on human health, the dose-response relationship, and adverse effects will be discussed.

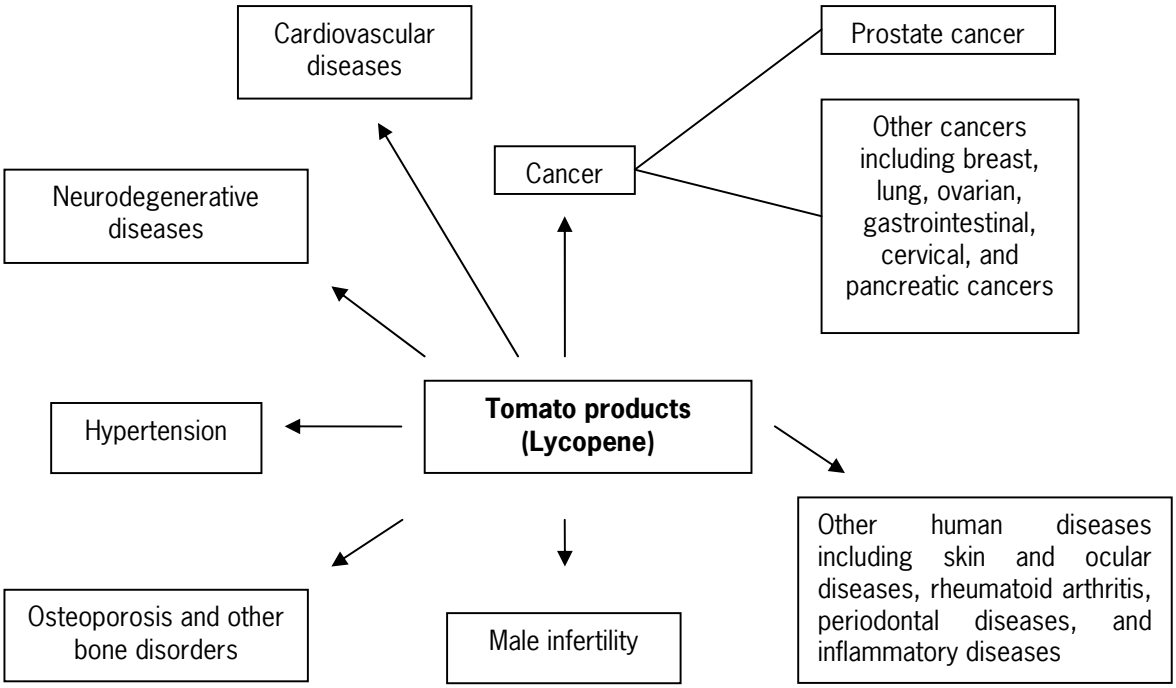

Figure 7. The role of tomato/tomato products (lycopene) in human health (based on review by Rao A.V. *et al.* (Rao, Ray et al. 2006))

3.6.1 Target Group

An important criteria to substantiate a health claim is that the subjects in the studies are representative for the target group for the claim in question. In the table of appendix IX a differentiation between studies with healthy subjects and studies with subjects suffering of a disease has been made. Four recently published studies concerning tomatoes/lycopene and cancer risk in healthy subjects are shown in that table (Porrini, Riso et al. 2005; Riso, Brusamolino et al. 2006; Riso, Visioli et al. 2006;

Zhao, Aldini et al. 2006). Two of those studies showed significant reduction in DNA damage, suggesting a decrease in cancer risk, after an intervention with tomatoes/lycopene (Porrini, Riso et al. 2005; Zhao, Aldini et al. 2006). Although studies with subjects who suffer from a disease are important to get more insight in the role of tomatoes/lycopene in human health, studies with healthy subjects are required for the health claim concerning tomatoes and oxidative stress, mentioned in this report.

3.6.2 Markers of oxidative stress

As mentioned previously, claims should be based on evidence related to markers that are linked to clearly defined and measurable outcomes and consistently modulated by the particular food component in rigorously controlled studies. If evidence is based on a marker of enhanced function, then an enhanced function claim can be made. If evidence is based on a marker of reduced risk of disease, then only in this case a reduction of disease risk claim can be made (Ashwell 2002). Figure 2 of chapter 1 shows this relation between the type of markers and the types of claims. Regarding oxidative stress, only an enhanced function claim can be made. Markers of oxidative stress should be based on the criteria discussed in chapter 1 of this report.

A wide variety of biomarkers for oxidative stress for humans are present. Functional assays, both *in vivo* and *ex vivo*, include various measures of DNA oxidation (oxidized DNA bases such as 8-OHdG, autoantibodies to oxidized DNA, modified comet assay), lipid oxidation (thiobarbituric acid-reactive substances, exhaled pentane/ethane, low density lipoprotein resistance to oxidation, F_2 isoprostanes), and protein oxidation (protein carbonyls) (Hwang and Kim 2007). DNA strand breaks which reflect the balance between DNA damage and repair, is usually measured in peripheral blood mononuclear cells (PBMC) or lymphocytes. The comet assay is a very sensitive method for measuring DNA strand breaks in individual cells to assess (Hwang and Kim 2007). This method is used in several important studies (Porrini, Riso et al. 2005; Riso, Brusamolino et al. 2006; Zhao, Aldini et al. 2006) investigating the relation between tomatoes/lycopene and oxidative stress.
One of the most commonly applied assays, for instance used in the study by Bose *et al.* (Bose and Agrawal 2007), for measuring lipid oxidation is the thiobarbituric acid reactive substances (TBARS) (Hwang and Kim 2007). However, there are several doubts about the validity of this test, because of interference problems. To minimize interferences with other compounds and therefore increase specificity, this test should be used in addition to HPLC. Another important measurement for lipid oxidation are F_2 isoprostane, for example used in a study by Riso *et al.* (Riso, Visioli et al. 2006). Evidence is increasing that isoprostanes play a causative role in carcinogenesis and artherogenesis (Hwang and Kim 2007).

The role of tomatoes, tomato products or lycopene in the development of prostate cancer has been studied relatively intensive. At this point the most predictive biomarkers for prostate cancer seem to be DNA damage and IGF (Bowen 2005). Considering the correlation between oxidative DNA damage in leukocytes and prostate tissue, DNA strand breaks of PBMCs or lymphocytes against oxidative challenge *ex vivo* seem to be an appropriate marker for the risk of prostate cancer (Ellinger, Ellinger et al. 2006). In contrast, the use of DNA strand breaks *in vivo* and the level of oxidized DNA bases in PBMCs are questionable as an appropriate marker for the risk of prostate cancer (Ellinger, Ellinger et al. 2006). Some authors have suggested that the plasma level of IGF-1 can be used as an early marker of the risk of developing cancer. In particular, increased blood levels of IGF-1 have been associated with increased risk of several common cancers such as prostate, breast (in premenopausal women), and lung (Riso, Brusamolino et al. 2006).

Despite the availability of the abovementioned techniques and markers, it still remains unclear how good these markers truly represent oxidative stress (Hwang and Kim 2007). Thus, a conclusive biomarker, satisfying the criteria stated in this report, can not yet be pointed out. Therefore, more

sensitive, specific methods should be developed that allow measurements of DNA, lipid, and protein adducts in humans exposed to oxidative stress.

3.6.3 Dose-response relationship

There are some animal studies conducted about the dose response relationship between tomato lycopene consumption and lipid peroxidation and antioxidant enzymes (Breinholt, Lauridsen et al. 2000; Chandra Mohan and Nagini 2003). However, these doses cannot be extrapolated to humans.
The intake of lycopene is usually evaluated on basis of food frequency questionnaires. In general the relationship between estimated intake and serum lycopene is very low (correlation coefficient = 0.1 – 0.34) (Clinton 1998). Lycopene intake in the United Kingdom was estimated 5.01 mg/day, and in the Netherlands 4.86 mg/day (O'Neill, Carroll et al. 2001). In the United States more than 80% of the lycopene consumed is derived from tomato products (Clinton 1998). However, in a study of Rao *et al.* the average daily dietary lycopene intake levels in Canada measured by a food frequency questionnaire, were estimated to be considerably higher, 25 mg (Rao, Waseem et al. 1998). Processed tomato products accounted for 50% of the total lycopene intake. Another study of Rao *et al.*, set up a recommended intake of lycopene of 35 mg daily that can be obtained by consuming two glasses of tomato juice or through a combination of tomato products (Rao and Agarwal 2000). With this dose, serum lycopene concentrations are significantly increased. The health effect of this increase in serum concentration lycopene is however, not investigated. Nothing is known about recommendations for fresh tomatoes intake. The ideal intake of lycopene is currently unknown, and certainly not for the intake of fresh tomatoes.
More research about the dose-response relationship between lycopene and health effects is needed. Dosage levels are important for a possible claim, but also for further long-term intervention studies. For a possible health claim on the product, long-term effects must be guaranteed, thus a long-term intervention is needed with fresh tomatoes containing a standardized concentration of lycopene. It is suggested that the length of the intervention period will be similar to the length of comparable studies, like the study of Gardner *et al.* In this parallel-design RCT with 192 adults, garlic was consumed 6d/wk for 6 months (Gardner, Lawson et al. 2007).

3.7 Pre-harvest

Tomatoes constitute almost the sole available dietary source of lycopene, a carotenoid with a high oxygen-radical scavenging and quenching capacity, thought to be responsible for the beneficial health effect. Tomatoes are also abundant sources of other antioxidant micronutrients, such as β-carotene, lutein, phytoene, phytofluene, γ-carotene, vitamin C, vitamin E and phenolic compounds. Several of these constituents may contribute to the health promoting properties of tomatoes. Tomatoes for example have been identified as the most important suppliers of phenols in the human diet, followed by corn and beans (Vinson, Hao et al. 1998).

The antioxidant contents of tomatoes depend on genetic and environmental factors (temperature, light, water availability, nutrient availability), the agricultural techniques used (cultivars, plant growth regulators, date of harvest, etc) and post-harvest storage and processing conditions. Two different studies (Lister 1999; Lenucci, Cadinu et al. 2006) reported however that there was no correlation between carotenoid concentration and antioxidant capacity in tomatoes. Since lycopene is purported to be such a strong antioxidant, responsible for the health effect of tomato, it is surprising that observed differences in lycopene among different cultivars is not coupled with differences in antioxidant capacity. Since the taste, color and nutrient qualities of tomatoes can also depend on their antioxidant contents, further insights into the factors likely to affect their composition should help to define the quality of tomatoes more clearly.

3.7.1 Concentration of health promoting compounds in fresh tomatoes

Lycopene

The lycopene content is the main factor that influences the color of red tomatoes. Thus, the color of tomato fruit is a fair indicator of lycopene concentration (Cox, Stushnoff et al. 2003). More sophisticated methods can identify the two occurring *cis* and *trans* isomers of lycopene, for example by using high-performance liquid chromatography (HPLC) (Khachik, Beecher et al. 1995). Guides for carotenoid analysis in foods, including extraction and detection methodologies, have been published (Rodriguez-Amaya and International Life Sciences 1999; Fish, Perkins-Veazie et al. 2002).

When turning from orange to red, fruit lycopene concentrations of between 32 and 43 mg/kg fresh weight (FW) have been measured. A total carotene concentration of more than 55 mg/kg was required for a satisfactory shade of red to develop, and lycopene then accounted for 90% of the carotenes (Dumas, Dadomo et al. 2003). The fresh tomato lycopene content can range from 43 to 181 mg/kg, and most of the values usually range between 55 and 80 mg/kg. The skins can contain about five times more lycopene (540 mg/kg) than the tomato pulp (110 mg/kg) (Dumas, Dadomo et al. 2003).

Other tomato compounds with potential health benefits

The mean values of vitamin C content of tomatoes range from 150 to 230 mg/kg raw edible part of the tomato and the normal range is from 84 to 590 mg/kg. These very large variations may be attributed to variations in the light intensity a few days prior to the harvest. In addition, it has been noted that the larger the fruit, the lower the vitamin C concentrations tends to be (Dumas, Dadomo et al. 2003).

For another tomato compound, vitamin E, studies have reported that, in unpeeled tomato fruit, the mean vitamin E content was 60 mg/kg dry weigh (DW) or 3 mg/kg FW, with 95% confidence limits of 35–100 mg/kg (Beecher 1998). The tocopherol content of tomato was found to be moderately high, ie 6.6 mg/kg at 95.4% moisture.

As far as phenolic compounds are concerned, 98% of flavonols detected in tomatoes, primarily as conjugates (quercetin and kaempferol), were found to occur in the skin and the total flavonol content of 20 fresh tomato varieties varied from 1.3 to 22.2 mg/kg. The levels of folate (0.13 mg/kg) and of quercetin (8 mg/kg) have been estimated (Beecher 1998). The kaempferol content was always <2 mg/kg of the fresh edible part of the fruit.

3.7.2 Genetic varieties

Lycopene concentrations in fruits depends on genetics and consequently the choice of cultivated variety is important. While almost all tomato varieties contain substantial amounts of total lycopene, significant variations among cultivars and types are evident.

Plant-breeding programs have used both traditional and molecular methods to enhance levels of lycopene and other carotenoids in fruits. Single-gene color-mutants of tomatoes were first documented in the 1940s, resulting from crosses with a normal canning tomato (*Lycopersicon esculentum*, Mill) and a wild species (*Lycopersicon esculentum*, Humb) to produce a high beta-carotene containing orange fruit (Ronen, Carmel-Goren et al. 2000). The genes and enzymes controlling carotenoid synthesis in tomatoes have been identified through cloning and molecular characterization (Ronen, Carmel-Goren et al. 2000). Nine genetic loci (15 alleles) controlling color pigmentation have been found and additional genes may exist (Sacks and Francis 2001). The crimson gene (og^c) has been bred into many of the commercial tomato processing materials that are currently used. This gene confers an inability to acquire beta-carotene at the same rate as normal red tomatoes (r^+) but increases the amount of lycopene that is formed. However, many of the other high-pigment genes are not used in breeding materials because they impart undesirable plant characteristics (i.e., poor germination, brittle stems) (Sacks and Francis 2001).

In a study evaluating lycopene content in 40 different tomato varieties (Kuti and Konuru 2005), the total lycopene content in fresh market tomato varieties (*L .esculentum* Mill) grown in the greenhouse ranged from 6 mg/kg to 48 mg/kg, while in those tomatoes grown in the field the content ranged from 4 mg/kg to 32 mg/kg. Generally, among the cluster and round tomato types (*L. esculentum* Mill), greenhouse-grown tomatoes consistently contained more total lycopene (mean = 30 mg/kg) than field-grown tomatoes (mean = 25 mg/kg). However, among the cherry tomato types (*L. esculentum* var cerasiforme) there was significantly more lycopene in field-grown (mean = 92 mg/kg) than in greenhouse-grown (mean = 56 mg/kg) fruits.

Cherry tomatoes

Cherry tomatoes are characterized by relatively high levels of antioxidants such as vitamin C, tocopherols, total phenols, and carotenoids, particularly lycopene. In several studies (Kuti and Konuru 2005; Lenucci, Cadinu et al. 2006), different cultivars of cherry tomatoes show a high variation in the content of lycopene. In the study of Kuti and Konuru the total lycopene content in cherry tomatoes (*L. esculentum* var cerasiforme) grown in the greenhouse ranged from 49 mg/kg to 64 mg/kg, while when the same tomatoes were grown in the field the content ranged from 74 mg/kg to 117 mg/kg. The study of Lenucci *et al.* reported a range of 43 mg/kg to 120 mg/kg for different cherry tomato cultivars when grown in the field. Similar variations in lycopene content, ranging from 50 to 110 mg/kg, have been reported in Hungarian varieties of tomatoes (Abushita, Daood et al. 2000). Variations between 20 and 70 mg/kg have also been reported in Indian cultivars (George, Kaur et al. 2004). It has been suggested that cherry tomatoes may be useful varieties for processing and for improvement of nutritional and health benefits in tomato breeding programmes (Kuti and Konuru 2005).

High-pigment tomatoes

High-pigment tomato hybrids are relatively new selections obtained by conventional plant-breeding programs finalized to increase lycopene content. In the study of Kuti and Konuru, a variability in lycopene content was found in high-pigment tomato hybrids ranging from 175 mg/kg to 253 mg/kg.

3.7.3 Environment and cultivation techniques

Effect of temperature

Lycopene synthesis and degradation in tomatoes is sensitive to air temperatures and light intensity. High temperatures (>32 °C) are known to suppress lycopene. The formation of lycopene depends on the temperature range and seems to occur between 12 and 32 °C. This process was found to be at an optimum between 16–18 and 26 °C in fresh tomato fruit harvested at the pink-ripe stage and left to ripen for several weeks (Türk, Seniz et al. 1993).

Effect of light

Lycopene synthesis has been identified as a phytochromeregulated response. Red light induces lycopene formation while far red light reverses it. It has been shown that brief red-light treatment of harvested mature green fruit stimulated lycopene accumulation 2.3-fold during fruit development (86.6 mg/kg at the redripe stage), compared with a dark control treatment (37.2 mg/kg), and that this could be reversed by subsequent treatment with far-red light (51.5 mg/kg) (Alba, Cordonnier-Pratt et al. 2000). This light-induced lycopene accumulation is regulated by fruit-localized phytochromes and is independent of ethylene biosynthesis. It is however shown that light is not required for the ripening of fruit picked at the breaker stage, as tomatoes will turn red even in complete darkness, but do not turn quite as red as those exposed to light (Dumas, Dadomo et al. 2003).

At favourable temperatures, the rates of synthesis of lycopene and carotene can be increased by illuminating tomato plants during the ripening of the fruit. Fruit exposed to direct sunlight during its development has higher carotene levels than shaded fruit (Dumas, Dadomo et al. 2003). However, the production of lycopene is inhibited by excessive sunlight. The best conditions are sufficiently high temperatures along with sufficiently dense foliage to protect the fruit from direct exposure to the sun. The carotenoid contents of fresh tomato fruit produced under glass, plastic tunnels and in the open field were compared (Lenucci, Cadinu et al. 2006). Fruit grown under a glass or plastic tunnel yielded a β-carotene content lower than in the open field. The lycopene content increased from glass to plastic tunnel to field, in that order. The level of intercepted light may have affected the carotenoid content, but interactions may also have occurred with the high temperatures occurring under protected growth conditions.

Light exposure is also favorable to vitamin C accumulation in the tomato fruit. Plants grown under high-light had an approximately twofold greater soluble phenols content than low-light plants. Since the formation of flavonol glycosides (eg kaempferol and quercetin) requires light, it is not surprising that these substances (to be found mainly in the skins of fruit) also benefit from the high light conditions (Dumas, Dadomo et al. 2003) .

Effect of water availability

Tomato response to water availability depended on type and cultivar with a decrease in lycopene found with decreased soil water in three cultivars, and an increase in lycopene seen in cherry or greenhouse-grown beefsteak types (Dumas, Dadomo et al. 2003). Using saline water (to 0.25% NaCl w/v) increased lycopene content of tomatoes (Krauss, Schnitzler et al. 2006). Use of other types of ions for salinity treatments (such as calcium or sulfate) has not been reported (Collins and Perkins-Veazie 2006).

Effect of mineral nutrients

Although potassium (K) and phosphorous (P) are needed for lycopene synthesis, response is dependent on rate, crop, germplasm, growing conditions, and growth stage. Extra soil potassium is needed to avoid yellow shoulder in processing tomatoes grown in California (Hartz 1999), implying a role for potassium in lycopene synthesis or degradation. Hydroponically grown tomatoes responded to increased rates of K and P with increased lycopene (20% to 30%) (Dumas, Dadomo et al. 2003). Fresh market tomatoes had little or no increase in lycopene with increased soil K rates in field experiments

(Fontes, Sampaio et al. 2000). Applications of P at high rates failed to increase lycopene levels in juice from processing tomatoes (Oke, Ahn et al. 2005).

Effects of growth and development regulators

Various growth and development regulators positively influence the tomato fruit carotene and vitamin C contents. But before planning to apply those substances to tomato fields, studies should be undertaken to test and confirm the feasibility of these interventions, and to evaluate the possible consequences in terms of the crop response and the hygienic quality of the fruit (Dumas, Dadomo et al. 2003). At room temperature, ethephon treatment enhanced the ripening of the fruit and ethephon combined with CPTA resulted in faster and greater lycopene accumulation. In field-grown tomatoes, gibberellic acid and cycocel (2-chloroethyl trimethylammonium 3-chloride) increased the β-carotene content of the fruit (Dumas, Dadomo et al. 2003).

Effect of stage in fruit development and ripening

The lycopene content can be said to be a good index to the level of maturation (Dumas, Dadomo et al. 2003). The results of numerous studies have indicated that the lycopene content tends to increase sharply towards the end of the ripening process. However, it is difficult to compare the results obtained because the cultivars used were different and the stages considered were often described subjectively.

In the Homestead cultivar (cv) fruit harvested and ripened at 22 °C, the carotenoids were analyzed at six stages of maturity. The lycopene content increased from 0.41 mg/kg at the breaker stage to 9 (pink stage), 22 (light-red stage) and 83 mg/kg at the red stage (Dumas, Dadomo et al. 2003). On tomato plants (cv Fireball) grown in a growth chamber (16 h light period at 24 °C and 8 h dark period at 18 °C, relative humidity 65%), the total carotenoids in the fruit increased constantly during the ripening process from 0.1 to 70 mg/kg (Trudel and Ozbun 1970). In tomato fruit (cv Moneymaker) grown in a greenhouse, a rapid fall in the chlorophyll content occurred at the onset of the mature-green stage and the chlorophyll had disappeared completely by the beginning of the pink stage (Rabinowitch, Budowski et al. 1975). During the same period of time, the fruit β-carotene content increased twofold. Lycopene and its colorless precursors, phytoene and phytofluene, began to accumulate after the breaker stage. At the ripe-red stage, lycopene constituted 95% of all the colored carotenoids and 73% of the total carotenoids. In the open field, the fruit β-carotene content increased regularly during ripening, whereas the lycopene content increased sharply between the stage pink and the stage red fruit.

The changes in the antioxidant contents at seven stages during vine and post-harvest ripening have been assessed in two genotypes (Normal Red and Crimson) of the tomato cv Moneymaker grown in a greenhouse. In vine-ripened tomatoes, the lycopene and β-carotene concentrations showed a gradual, linear increase during the ripening process, whereas in post-harvest-ripened fruit, the lycopene and β-carotene levels followed an exponential trend. At the end of the experiments, the lycopene and β-carotene concentrations (roughly 125–130 mg/kg and 12 mg/ kg respectively) in post-harvestripened tomatoes were almost twice as high as the values reached in vine-ripened tomatoes (roughly 75–80 mg/kg and 5–7mg/kg respectively) having the same colour ($a*/b*$) index. Appropriate post-harvest storage conditions can, therefore, give tomatoes a higher lycopene content (Dumas, Dadomo et al. 2003).

Fruit bruising at the breaker stage can decrease (−37%) the total carotenoids present in the locular tissue of the fruit when it reaches the ripe stage (Moretti, Sargent et al. 1998).

In conclusion, some of the factors involved in the development of various antioxidants seem to be contradictory. Water shortage during cropping might enhance the fruit vitamin C content and possibly reduce the lycopene content. Likely, direct sunlight may favor the accumulation of vitamin C and phenols in the fruit, whereas lycopene may develop more readily in fruit protected by crop foliage. Little information has been published about: N and P/lycopene and water/lycopene. To asses lycopene variability, it is necessary to know more about the effects of water on antioxidants in tomatoes,

because water is a main factor of tomato quantitative and qualitative production. As far as the effects of fertilization are concerned, the data available are either rare or not very reliable or applicable. As a result, at present, it seems difficult and hazardous to define optimum growth conditions for maximizing the biosynthesis and storage of lycopene in the tomato fruit. It would be of great interest to have more data to help to understand more clearly how agronomic factors and techniques affect the canopy characteristics and interfere with the effects of light and temperature, which, along with the genetic background, are the main determinants really responsible for the accumulation of lycopene during the fruiting period.

3.8 Post-harvest

The processing, handling, storage and ripening of tomatoes are processes that can influence the lycopene content of the product and the bioavailability of lycopene in humans. Therefore it is important to pay attention to these aspects.

3.8.1 Post-harvest ripening

Ripening of tomatoes has been widely studied with the main objective to increase the shell life. In the study of Giovanelli, seven different ripening stages were compared from mature-green to full red. The variation in the antioxidant content was evaluated in two tomato varieties. Lycopene synthesis showed the highest increase during ripening and its content was not linearly related to color changes. The lycopene and the ß-carotene concentrations in post-harvest-ripened tomatoes were almost twice the value compared to vine-ripened tomatoes having the same color. (Giovanelli, Lavelli et al. 1999).
Lycopene accumulation during post-harvest ripening followed an exponential rate up to high concentration values in the very last period of ripening, before over-ripening symptoms became evident. Higher values were measured in the post-harvest ripened tomatoes than in the vine-ripened tomatoes. It must be mentioned that also ascorbic acid and other phenolic compounds had higher values in the post-harvested tomatoes than the vine-ripened tomatoes.
In another study about the lycopene content of tomatoes, maturity at harvest, ethylene treatment, and storage time all played a role in the lycopene accumulation in tomatoes during ripening. In general, tomatoes that were at the breaker stage (10% red) at harvest had a greater accumulation of lycopene during room temperature storage than tomatoes that were green at harvest and received ethylene to stimulate ripening (Thompson, Marshall et al. 2000).
The results are controversial, because another study reported that tomatoes ripened on vine had significantly more lycopene, ß-carotene, soluble and total solids, higher color intensity and were firmer compared to post-harvested tomatoes. (Arias, Lee et al. 2000) This study shows precisely the opposite results from other data.
Definitely, more research is needed about this topic. The accumulation of lycopene during ripening is a very interesting method to increase the lycopene content of the tomatoes. At present, the results are inconsistent, maybe because of different measurement methods or different tomato varieties.

3.8.2 Storage

The most important factor contributing to degradation of lycopene is the availability of oxygen during storage. Storage conditions for tomatoes are advised with low oxygen, low light and low water content (Shi and Maguer 2000). In general, storage temperatures at 20°C to 30°C stimulate lycopene production, whereas temperatures of 5°C or less reduce lycopene. Ethylene, a plant hormone, promotes ripening, cell wall softening, and subsequent lycopene synthesis in tomatoes. Ethylene blockers like carbon dioxide and 1-MCP (1-Methylcyclopropane) are used to increase the shell life of tomatoes. These blockers slow or block ethylene triggered lycopene formation (Collins and Perkins-Veazie 2006).
In another study, 1-MCP only delayed the onset of ripening-related changes and did not significantly alter final values for measures of firmness, color, polygalacturonase activity, lycopene and chlorophyll contents at a particular storage temperature (Mostofi, Toivonen et al. 2003). Polygalacturonase activity plays a role in cell wall degradation and fruit softening.
Thus, such kind of ethylene blockers delay the ripening process with a few days, but the lycopene content in total does not change significantly.
While *trans* to *cis* isomerization typically occurs during processing, the storage of processed foods favors reversion from *cis* to *trans*, because of the relatively unstable state of *cis* isomers compared with the *trans* isomer (Xianquan, Shi et al. 2005). Compared to the other *cis*-isomers, the 5-*cis*-isomer form is the most stable, also during storage. Thus, a shorter storage period for processed tomato products is suggested for the bioavailability of lycopene.

The majority of the literature about storage is about the lycopene degradation in tomato-based products, not about storage of fresh tomatoes. Therefore, more research about the effect of storage on the lycopene content in fresh tomatoes is advised.

3.8.3 Effects of processing

Effect of temperature

As described in the chapter 'Bioavailability', it is thought that *cis*-lycopene is more bioavailable than *trans*-lycopene because *cis*-isomers are more soluble in bile acid micelles and may be preferentially incorporated into chylomicrons (Boileau, Merchen et al. 1999). Heat treatment promotes isomerization of lycopene in tomatoes, from *trans* towards *cis* isomers, especially 9-*cis*-isomers. The degree of isomerization depends on the intensity and the duration of the heating (Shi and Maguer 2000). This means that lycopene in processed tomato products are more bioavailable than lycopene in fresh tomatoes because of the isomer forms. *Trans*-lycopene is the most predominant isomer in fresh tomatoes.

As shown in table 2, heating tomato juice for 7 minutes at 90˚C and 100˚C resulted in a 1.1 and 1.7 % decrease in lycopene, respectively. These temperatures are the normal home cooking temperatures. At higher temperatures and longer heating times greater losses are observed (Shi and Maguer 2000). Lycopene levels decreased significantly in fried sampling, this are temperatures of 150˚C or more (Sahlin, Savage et al. 2004). These results suggest that length and intensity of heating are critical factors for controlling the degradation of lycopene. The study of Graziani reports that significant decreases in lycopene content of the tomato products are only seen after 2 hours of heating (Graziani, Pernice et al. 2003). Thermal treatment led to isomerization of all-*trans* and 13-*cis* lycopene into 9-cis isomer, at 90˚C. The concentration of the 9-*cis* isomer was significantly increased during the heating treatment (Ax, Mayer-Miebach et al. 2003).

Table 2. Lycopene loss rate in tomato juice during heating (Shi and Maguer 2000)

Heating temperature (°C)	Lycopene loss (%)		
	Heating time 1 min	Heating time 3 min	Heating time 7 min
90	0.6	0.9	1.1
100	0.9	1.4	1.7
110	2.2	3.2	4.4
115	2.7	4.5	7.0
118	3.7	6.0	9.1
121	4.6	7.3	10.6
124	5.5	8.5	12.5
127	6.5	9.9	14.6
130	7.4	11.5	17.1

Effects of peeling

The epidermal area of tomatoes (skin and outer pericarp tissue) contains more than 80 to 90% of the total lycopene in tomatoes (Shi and Maguer 2000). This means that peeling the tomato severely decreases the lycopene content and thus intake.

3.8.4 Effects of food matrix

Lycopene bound in the matrix is less bioavailable. Cooking or fine grinding of foods could increase bioavailability by disrupting or softening plant cell walls and disrupting lycopene-protein complexes. Thus processes like cooking and chopping are convenient ways to make lycopene more accessible (Shi and Maguer 2000). Van het Hof also concluded that the intactness of the cellular matrix of tomatoes determined the bioavailability of carotenoids and that matrix disruption by mechanical homogenization and/or heat treatment enhances the bioavailability (Hof, de Boer et al. 2000).

Much more *trans*-lycopene, than *cis*-lycopene is present in fresh tomatoes. This supports that the matrix seems to contribute to the stability of the isomers.

3.8.5 Effects of oil medium

Lycopene bioavailability from tomato products seems significantly higher when consumed in combination with oil compared to the consumption of the same product without oil. Stahl and Sies investigated (Stahl and Sies 1992) the uptake of lycopene from processed (boiled with 1% corn oil for 1 h) and unprocessed tomato juice in humans. The bioavailability of the tomato juice cooked with oil was better compared to the unprocessed juice. It is assumed that heating the tomato juice in the presence of corn oil converts lycopene from *trans* to *cis* isomers, thereby increasing its absorption by the body (Shi and Maguer 2000).

A more recent randomized crossover trial with two diets, one diet was high in refined olive oil and the other high in carbohydrate and low in refined olive oil; both diets contained the same basic foods and a controlled carotenoid content high in lycopene. This study found no difference in serum lycopene between the two diets. However, a diet high in olive oil and rich in lycopene may decrease the risk of coronary heart disease by improving the serum lipid profile compared with the high-carbohydrate diet (Ahuja, Pittaway et al. 2006).

In many other studies with extra virgin olive oil, serum lycopene levels in the body increased after tomato consumption with olive oil. (Ahuja, Pittaway et al. 2006). These results may come from the type of fats present in the oil. Another possible explanation is that the oil mixed with the tomato(Stahl and Sies 1992; Fielding, Rowley et al. 2005), rather than present in the overall diet (Ahuja, Pittaway et al. 2006) results in a positive outcome.

The study of Fielding . concluded that the addition of olive oil to diced tomatoes during cooking greatly increases the absorption of lycopene. There was an 82% increase in plasma *trans*-lycopene ($P < 0.001$) and a 40% in *cis*-lycopene ($P = 0.002$) concentrations in the 11 subjects who consumed tomatoes cooked for 10 minutes in olive oil. There was no significant change in *trans*-lycopene (P=0.684) and a 15% increase in *cis*-lycopene ($P = 0.007$) concentrations in 12 subjects consuming tomatoes cooked without olive oil (Fielding, Rowley et al. 2005).

The results highlight the importance of cuisine (i.e how a food is prepared and consumed) in determining the bioavailability of dietary carotenoids such as lycopene.

3.8.6 Effect of dietary fibers

Various types of dietary fiber were found to reduce the bioavailability of carotenoids in foods. In the study of Rock and Swendseid a significant decrease in plasma β-carotene concentration pectin was found (Rock and Swendseid 1992). Another study with an antioxidant supplement consisting of β-carotene, lycopene, lutein, canthaxanthin and α-tocopherol indicated that a dietary fiber supplementation decreases the antioxidative effect of a supplement consisting of carotenoids and α-tocopherol in LDL, an effect that is likely to be mediated by a reduced bioavailability of these antioxidants in the gut (Hoffmann, Linseisen et al. 1999). There are no studies found with effects of dietary fibers on lycopene in tomatoes or lycopene solely.

In conclusion, lycopene remains relatively stable during food processing. *Trans* to *cis* isomerization may happen during processing, especially in the presence of oil. *Cis*-isomers are better absorbed by humans compared to *trans*-isomers. Peeling tomatoes is not advisable for lycopene intake, because 80 to 90% of the lycopene is present in the epidermal area of the tomato. The inhibiting effect of dietary fibers on lycopene is not clear yet. Future research should focus on the inhibition of the fibers on lycopene in tomatoes solely. During storage is lycopene less stable compared to processing. The general recommendations for storage are low oxygen, low light and low water. Most research on storage is in tomato products, not in fresh tomatoes. The ripening process of the tomatoes seems very important for the lycopene concentrations, especially in the last phase. The ripening of tomatoes and the lycopene accumulation during vine or post-harvest is not fully understood, thus more research

is needed. Furthermore, research is needed about tomatoes with a prolonged shell life, to investigate the accumulation of lycopene during post-harvest ripening.

Conclusion

This paper evaluated which compounds, present in fresh food or vegetables, could aid the certification of a fresh fruit or vegetable for a health claim within a limited time. Out of a selection of different compounds from six different chemical groups (Flavonoids, Tannins, Organosulfur compounds, Terpenoids, Vitamins and minerals) lycopene, mainly present in tomatoes, was chosen as the most promising phytochemical. Selection of lycopene was based on beforehand formulated criteria, such as reliable clinical trial data and possibility to produce the fruit or vegetable matrix the Netherlands.

Dietary intake of tomatoes and tomato products containing lycopene have been mainly associated with oxidative stress. Therefore it is expected that a claim on the target function "oxidative stress" is the most reasonable in the near future.
Present knowledge about the pharmacokinetics of lycopene, mechanism behind the health beneficial effect of tomatoes/lycopene, synergy, pre- and post-harvest conditions, dose-response relationship, and adverse effects of tomatoes/lycopene, which is essential for the certification of a tomato for a health claim, have been addressed in the second part of this literature study.

Studies on the pharmacokinetics of lycopene clearly show that lycopene is readily bioavailable. The absorption process of lycopene in the GI-tract is well understood compared to other pharmacokinetic factors. However, the effects of the food matrix on lycopene absorption and the fate of all-*trans*-lycopene and its *cis*-isomers in the gut are subjects for further research. Matrix effects and other events in the upper GI-tract are however difficult to study in humans. Therefore, the use of *in vitro* digestion models and for example *in vivo* ferret models is suggested to gain further insight on this topic.
Long term lycopene status, in both blood and tissues, needs to be further explored to investigate the effects of a diet rich in tomatoes. Also tissue specific biological actions are of interest.
Hardly anything is known about metabolism and excretion of lycopene. More research in this field may yield new metabolites of lycopene and provide information on possible mechanisms. Concerning the *cis*-isomers and all-*trans*-confirmation of lycopene, the possible *cis*-isomer formation *in vivo*, the metabolism and introversion of isomers, and biological effects or functions of specific isomers are of interest for further investigation.
Though many aspects of lycopene pharmacokinetics are still unclear, research on this topic is not necessarily relevant for the application of a health claim. Further research could, however, reveal information on possible mechanisms and sites of action, and in this way provide information on promising health effects.

Mechanistic studies in animals and to a lower extent cell line studies have given insight in the numerous health beneficial mechanisms in which lycopene is involved, either by direct anti-oxidative activity or by enhancing or inhibiting pathways involved in carcinogenesis. Probably, a combination of several of these mechanism, rather than a single mechanism, cause the protective effect of lycopene on health.
It should be taken into account that most of the knowledge about lycopene on health is obtained from animal and cell line studies, data from clinical trials are difficult to obtain. Moreover, many results are derived from studies using lycopene supplementation, instead of administering tomato products. Therefore, more research with fresh tomatoes is needed. Although it is generally accepted that lycopene is the main health beneficial compound of tomatoes, it is still unclear whether the health effects are caused by lycopene itself, or by one of its derived metabolites. Hence, further research in this field is required.

A wide variety of biomarkers for oxidative stress for humans are present. Oxidative stress is a biomarker for cancer risk. Functional assays, both *in vivo* and *ex vivo*, include various measures of DNA oxidation, lipid oxidation, and protein oxidation. DNA damage (comet assay) and lipid oxidation (TBARS and F_2 isoprostane) are the most commonly used biomarkers in this field. At this point the most predictive biomarkers for prostate cancer seem to be DNA damage and insulin-like growth factor (IGF) (Bowen 2005). However, despite all these techniques and markers, it remains unclear what these measurements truly represent. Thus, a good biomarker does not exist yet. More sensitive, specific methods should be developed that allow measurements of DNA, lipid, and protein adducts in humans exposed to oxidative stress.

It is still questionable what the ideal lycopene intake range for a health effect is. Dosage levels are important for the potential health claim, but also for further long-term intervention studies. For a health claim on a food product, long-term effects must be guaranteed. Thus a long-term intervention with fresh tomatoes containing a standardized concentration of lycopene is needed. The duration of such a long-term intervention should be that long, that health effects over a long-term period can be observed. It should be considered, dose-response relationship investigated in animal studies cannot be extrapolated to humans. Thus, more research concerning dose-response relationship is needed, especially in human studies.

The antioxidant contents of tomatoes depend on genetic, on environmental factors (temperature, light, water availability, nutrient availability), the agricultural techniques used (cultivars, plant growth regulators, date of harvest, etc), post-harvest storage, and processing conditions.
Water shortage during cropping might enhance the fruit vitamin C content, but possibly reduces lycopene content. Likely, direct sunlight may favour the accumulation of vitamin C and phenols in the fruit, whereas lycopene may develop more readily in fruit protected by crop foliage. Little information has been published about the relation between the nutrients N and P and lycopene.To asses lycopene variability, it is necessary to know more about the effects of water on antioxidants in tomatoes, because water is a main factor of tomato quantitative and qualitative production. As far as the effects of fertilization are concerned, the data available are either rare or not very reliable or applicable. As a result, at present, it seems difficult and hazardous to define optimum growth conditions for maximizing the biosynthesis and storage of lycopene in the tomato fruit. It would be of great interest to have more data to help to understand more clearly how agronomic factors and techniques affect the canopy characteristics and interfere with the effects of light and temperature, which, along with the genetic background, are the main determinants really responsible for the accumulation of lycopene during the fruiting period.

The processing, handling, storage and ripening of tomatoes can influence the lycopene content of the tomato and thus the bioavailability of lycopene in humans.
Lycopene remains relatively stable during food processing. *Trans* to *cis*-isomerization may occur during processing, especially in the presence of oil. *Cis*-isomers are better absorbed by humans compared to *trans*-isomers. Peeling tomatoes is not advisable for lycopene intake; 80 to 90% of the lycopene is present in the epidermal area of the tomato. The inhibiting effect that dietary fibers cause on lycopene bioavailability is not yet clear. Future research should focus on this inhibiting effect of fibers on lycopene bioavailability. During storage is lycopene less stable compared to during processing. The general recommendations for storage of tomatoes are low oxygen, low light and low water. Most research on storage is in tomato products, not in fresh tomatoes. The ripening process of the tomatoes seems very important for tomato lycopene concentrations. There is a difference between vine and post-harvest ripening. Most studies in this field show a higher lycopene content when tomatoes are harvested at breaker stage and left for post-harvest ripening. However, results are contradictory, thus more research is needed. Furthermore, research is needed about tomatoes with a prolonged shell life, to investigate the accumulation of lycopene during post-harvest ripening.

Concerning adverse effects of tomatoes, only one case of slight reddishness of the skin was observed. Consumption of two liters of tomato juice a day for several years caused this skin colorization. This colorization however, disappeared after stopping the excessive lycopene intake. Until now, no adverse effects of lycopene supplements have been identified. Based on animal studies, the highest acute dose with no adverse effects was 5000 mg/kg body weight and 4500 mg/kg body weight for chronic and sub-chronic toxicity; extrapolated to humans this dose is 270 mg/day. Based on these and other results, lycopene supplementation is generally recognized as safe by the FDA.

In conclusion, there seems to be sufficient evidence to start an application procedure for a health claim at the moment. A claim on the target function oxidative stress, rather than on a disease, is the most reasonable for the near future. A reduction of oxidative stress is assumed to prevent or delay the occurrence of chronic diseases like cardiovascular diseases, cancer, and probably osteoporosis.
The actual EU certification of tomatoes as fresh functional food will need some additional research. Like the study by Gardner *et al.*, a long-term and large valid clinical trial should be performed using fresh tomatoes as intervention and a subject group that is representative for the target group of the claim. Validity of the study used to substantiate the health claim should be based on the legislation of ILSI. In addition, to actually introduce the tomatoes as fresh functional foods on the market, more knowledge about pharmacokinetics, mechanism, synergy, pre- and post-harvest conditions and dose-response relationship is needed/suggested. Before certification for the health claim is approved, it is suggested to carefully consider communication with the consumer and labeling formulation/regulation, including for instance the method of preparation for obtaining an optimal healthy product. Furthermore, when introducing the fresh functional tomato on the market it should be addressed that such a product is only relevant for health promotion when consumed in an overall healthy diet and lifestyle.

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Appendices

Appendix I

Plant compound: Selenium
Database(s): Medline
Search: "Selenium"[MeSH] AND/OR vegetables AND/OR health
Limits: English, Human study, Randomized Controlled Trial, No pilot studies

Author, year, location; intervention length and research design	**Intervention (dose, chemical form)**	**Subjects (age, gender, special properties/disease)**	**Study group**		**Bioavailability (if information available)**	**(Intermediate of) endpoints**	**Notable results**
			Intervention group (n)	**Control group (n)**			
You et al. 2006, China, 7.3 yr, RCT(You, Brown et al. 2006)	Selenium from yeast (37,5 µg twice daily) (together with Vitamin C and E)	Both sexes, 35-64 years	1677	1688	Not available	Prevalence of dysplasia or gastric cancer. Prevalence of severe chronic atrophic gastritis, intestinal metaplasia, dysplasia or gastric cancer. Average histologic severity score.	No statistically significant favorable effects were seen for this combination of vitamins and selenium on precancerous gastric lesions or on gastric cancer incidence
Hurwitz et al., 2006, USA, 9 months, RCT(Hurwitz 2007)	High selenium yeast supplementation (200 µg daily)	Age mean 40.5 Both sexes, HIV-1-seropositive persons	141	121	Yes, serum selenium concentrations increases significantly	Levels of HIV-1- viral load	Significant decrease in HIV-1-viral load in intervention group, which predicted increased CD4 count
Stranges et al, 2005, USA, 4.5 yrs (+7.6 yrs follow-up), RCT(Stranges, Marshall et al. 2006)	Selenized yeast (200 µg daily)	Both sexes, history of nonmelanoma skin cancers, free of CVD at baseline	504	500	Yes, significant change in plasma selenium concentrations	All cardiovascular disease, myocardial infarction, stroke, all cardiovascular disease mortality	Not significantly associated with any of the cardiovascular disease endpoints
Rayman et al, 2005, UK, 2 yrs, RCT(Stranges, Marshall et al. 2006)	Selenized yeast 100, 200, 300 µg daily	Age 60-74, both sexes, healthy volunteers	Respectively for 100, 200 and 300 µg 121, 127, and 127	126	Plasma selenium was significant increased in intervention groups	POMS-BI questionnaires for mood and quality of life	No significant difference measured between doses or with control
Hoenjet et al. 2004, the Netherlands, 21 wk, RCT(Hoenjet 2004)	Selenium supplement (200 µg daily) (and vitamin E,C and Coenzyme Q10)	Men, patients with hormonally untreated carcinoma of the prostate	36	34	Yes, significant increase in plasma levels in intervention group over the 21 weeks	Serum levels of PSA Hormone levels (testosterone, dihydrotestosterone, luteininzing hormone and sex hormone binding globulin)	No statistically significant differences seen in serum PSA levels or hormone levels for this combination of supplements

Duffield Lillico et al. 2003, USA, 4.5 yrs (follow up 6.4 yrs), RCT(Duffield-Lillico, Dalkin et al. 2003)	Selenized yeast (200 µg daily)	Men, history of basal cell or squamous cell carcinomas of the skin	457	470	Yes, significant change in plasma selenium concentrations	Overall incidence of prostate cancer	Significant decrease in prostate cancer incidence only for those with a baseline PSA level ≤ 4 ng/ml and with baseline selenium concentrations in the lowest two tertiles.
Sieja et al., 2002, Poland, 3 mo, RCT(Sieja 2004)	Selenium supplement (200 µg daily)	Women, patients with ovarian cancer undergoing chemotherapy	31	31	Yes, selenium concentrations in plasma and hair were significantly higher after 2 and 3 months in intervention group	GSH-Px in erythrocytes and MDA in serum. GSH-Px can protect the cells against oxidative damage. (MDA) is the final product of lipid peroxidation.	GSH-Px increase in erythrocytes in intervention group and a decrease of MDA in serum in intervention group
Clark et al, 1998;USA,4.5 yrs (follow-up 6.4), RCT (only abstract)(Clark 1998)	Selenium supplement 200 µg daily	Men, 18-80 yr, history of basal cell or squamous cell carcinomas of the skin	421	422	Not available	Now especially is studied the prostate cancer incidence with the same data base as Clark et al, 1996	Significant reduction in prostate cancer incidence during 1983-1993
Yu et al,1997; China, 4 yrs, RCT (abstract only)(Yu, Zhu et al. 1997)	Selenized yeast tablet (200 µg daily)	Both sexes, HbsAg-positive persons	113	113	Not available	Primary liver cancer development	The incidence is lower in intervention group but not significant
Clark et al, 1996; USA, 4.5 yrs (follow-up 6.4), RCT (Clark, Combs et al. 1996)(only abstract)	Selenium supplement 200 µg daily	Both sexes, 18-80 yr, history of basal cell or squamous cell carcinomas of the skin	656	656	Not available	The primary end points are the incidences of basal and squamous cell carcinomas of the skin. The secondary end points are all-cause mortality and total cancer mortality, total cancer incidence, and the incidences of lung, prostate, and colorectal cancers	Not significant for primary endpoints, significant for secondary endpoints.
Blot et al, 1995; China; 5.25 yrs, randomized trial(Blot, Li et al. 1995)	Selenized yeast (50 µg daily) in combination with □-carotene and α-Tocopherol	Both sexes, 40-69 yr, area with chronically low intake of several nutrients	30.000 (also other nutrient combinations)	-	Not available	Total and cancer mortality	Significant reductions in total and cancer (especially esophagus and stomach) mortality, reductions were greater in women and ≤ 55 yr ns

Appendix II

Plant compound: Folate
Database(s): Medline
Search: "Folate"[MeSH] AND/OR vegetables AND/OR health
Limits: English, Human study, Randomized Controlled Trial, No pilot studies

Author, year, location; intervention length and research design	**Intervention (dose, chemical form)**	**Subjects (age, gender, special properties/disease)**	**Study group**		**Bioavailability (if information available)**	**(Intermediate of) endpoints**	**Notable results**
			Intervention group (n)	**Control group (n)**			
Moens et al, 2007, Belgium, 6 wk, double-blind crossover trial with a 2-week washout.(Moens, Claeys et al. 2007)	High dose of folic acid supplementation (10 mg/d) *The plasma folate levels achieved in this study cannot be obtained by dietary fortification with folic acid*	Both sexes, normo- and hyperhomocysteinemia	40	40	Yes, plasma folate was measured	Plasma folate, total homo-cysteine and its subtypes Flow Mediated Dilution, and nitroglycerin-mediated dilation	Folic acid increased FMD in both normo- and hyper-homocysteinanemic groups. High-dose folic acid improves endothelial function in post-AMI patients, independent from homocysteine status.
Brouwer et al, 1999;Wageningen, 4 wk, RCT(Brouwer, van Dusseldorp et al. 1999)	dietary folate (350 µg /d) folic acid (250 µg/d)	Age18-45, both sexes	dietary folate: 23 folic acid: 22	placebo: 22	Yes, vegetables and citrus fruits relative to folic acid, 60% based on tHcy conc, 78% based on plasma folate conc, 98% based on red blood cell folate	plasma folate (wk 0,2,4) red blood cell folate (wk 0,2,4) plasma Hcy (wk 0,2,4)	Significant decrease in plasma Hcy compared to baseline in the dietary folate group and folic acid group

Appendix III

Food Product: Brassica vegetables (Glucosinolates)
Database(s): Medline
Search: "Glucosinolates"[MeSH], "Isothiocyanates" [Mesh] AND/OR Brassica vegetables AND/OR health
Limits: English, Human study, Randomized Controlled Trial, No pilot studies

Author, year, location; intervention length and research design	**Intervention (dose, chemical form)**	**Subjects (age, gender, special properties/disease)**	**Study group**		**Bioavailability (if information available)**	**(Intermediate of) endpoints**	**Notable results**
			Intervention group (n)	**Control group (n)**			
Fowke et al., 2006, USA, 2 x 4 wk, randomized cross-over desgin(Fowke, Morrow et al. 2006)	Brassica vegetables 218 g daily Or Micronutrient (Centrum® daily) and Fiber (1,5 g daily)	Both sexes, colon adenoma patients	20 (brassica vegetables)	20 (micronutrient and fiber)	Not available	Urinary F2-isoprostane,	F2-isoprostane significantly decreased during intervention compared with baseline or the micronutrient and fiber intervention. F2-isoprostane level is a stable biomarker for systemic oxidative stress
Kensler et al. 2005, China, 2 wk, RCT + placebo(Kensler, Chen et al. 2005)	Drinking hot infusions of 3-day-old broccoli sprouts	Age 25-65, both sexes, healthy but at high risk for development of hepatocellular carcinoma, due to consumption of aflatoxin-contaminated foods, and are exposed to high levels of phenanthrene, a sentinel of hydro-carbon air toxins	100	100	Yes, but highly variable, measurements of urinary levels of di-thiocarbamates (sulforaphane metabolites) indicated striking interindividual differences in bioavailability	Aflatoxin-N^7-guanine Trans, *anti*-Phenanthrene tetraol	No overall difference between intervention groups, only an inverse association for aflatoxin-DNA adducts and dithiocarbamates in individuals receiving broccoli sprout glucosinolates. Trans, anti-PheT, was detected in urine of all participants and showed an inverse association with dithiocarbamate levels.
Cashman,1999, USA; 3 wk, RCT cross over design(Cashman, Xiong et al. 1999)	Cooked Brussels sprouts (300 gr daily)	Men, healthy volunteers	5	5	Not available	Human flavin-containing monooxygenase activity was measured by determining the levels of urinary TMA and TMA N-oxide	Postive and significant decrease in the Human flavin-containing monooxygenase activity in sprouts group, this may have consequences for metabolism of other xenobiotics or dietary constituents
Verhagen et al. 1997, Netherlands, 1 wk, RCT(verhagen 1997)	Cooked Brussels sprouts (300gr daily)	Both sexes, healthy volunteers	5	5	Not available	excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG)	Positive in men, not significant. Reduction of 8-oxodG was found in men, is a biomarker for oxidative DNA-damage in whole body
Bogaards et al, 1994, Netherlands, 3 wk, RCT(Bogaards 1994)	Cooked Brussels sprouts (300gr daily)	Men, health volunteers	5	5	Not available	α-class glutathione S-transferase levels	α-class glutathione S-transferase levels were elevated by a factor of 1.4, this reflects GST-a induction in tissues such as liver and small intestine under non-toxic conditions.

Appendix IV

Plant compound: quercetin
Database(s): Medline
Search: "quercetin"[MeSH]
Limits: English, Human study, Randomized Controlled Trial

Author, year, location; intervention length and research design	Intervention (dose, chemical form)	Subjects (age, gender, special properties/disease)	Study group			Bioavailability (if information available)	(Intermediate of) endpoints	Notable results
			Intervention group (n)	Control group (n)				
Boyle et al. 2000, Scotland; 6 wks; Single-blind, placebo controlled trial. (Boyle, Dobson et al. 2000)	500 mg rutin in tablet (quercetin-3-O-betarutinoside)	Female volunteers Age 18-48 Healthy, prediet low in flavonoids, 48 hrs washout period	8	8 Placebo containing glucose		Plasma quercetin levels increased	Plasma antioxidant, quercetin, kaempferol, isorhamnetin, decreased pyrimidine, DNA breakage, MDA	Positive and sign for antioxidants, pos. trend for total antioxidant status, no change on base oxidation
Conquer et al, 1998, Canada/Italy; 28 days; Double-blind RCT; (Conquer, Maiani et al. 1998)	Capsule with: 1.0 gr quercetin, 1.0 gr bioflavonoids, 200 mg rutin, 200 mg bromelain	Healthy men/women from Canada, age 42 (mean)	13	14 Capsule with rice powder			Plasma quercetin, plasma falvonoids CVD risk factors	Pos, sign increase in plasma quercetin; no difference in CVD risk factors; no difference in platelet aggregation
McRae et al, 2006, US; 6 wks; rand double-blind, crossover study. (MacRae and Mefferd 2006)	Free radical scavenger (FRS) cocktail with or without quercetin (300 mmg/serving). 2 capsules/day	12, elite cyclists, healthy male	6	6			Endurance exercise performance	Sign improvement of performance after quercetin FRS cocktail, compared to FRS without quercetin
Shoskes et al, 2006 US; 1 month; rand. Placebo controlled trial. (Shoskes, Lapierre et al. 2006)	Capsule with: 480 mg curcumin, 20 mg quercetin	43 dialysis patients directly after renal transplantation surgery	High dose: 2 capsules/day	Low dose: 1 capsule/ day, 1 placebo	2 placebo capsules		Early graft function (EF) Delayed graft function: - serum creatinine - acute rejection rate - tremor	EF increased at both doses (P=0.013) - Serum creatinine sign decreased Rejection in high dose group 0% compared to low dose group

Food product: apple (quercetin)
Database(s): Medline
Search: "malus" [MeSH] AND "quercetin" [MeSH]
Limits: English, published in the last 5 years, Clinical Trial, Randomized Controlled Trial, No pilot studies

Author, year, location; intervention length and research design	Intervention (dose, chemical form)	Subjects (age, gender, special properties/disease)	Study group			Bio-availability (if information available)	(Intermediate) endpoints	Notable results
			Intervention group (n)	Control group (n)				
Enomoto et al, 2006, Japan; 4 wks; double-blind, rand, placebo controlled arm study	Apple juice, 1 bottle/day	33 Subjects with persistent allergic rhinitis for longer than 3 yrs Age 15 - 65	11 High dose: 200 mg polyphenols	11 Low dose: 50 mg polyphenols	11 Control, no polyphenols		Sneezing attacks, Nasal discharge, Swelling of the nasal turbinate	Pos sign results, especially for high dose group.

Appendix V

Food product: Garlic (Allicins)
Database(s): Medline
Search: "Garlic"[MeSH] NOT "Alopecia"[MeSH]
Limits: English, published in the last 5 years, Clinical Trial, Randomized Controlled Trial, No pilot studies

Author, year, location; intervention length and research design	**Intervention (dose, chemical form)**	**Subjects (age, gender, special properties/disease)**	**Study group**		**Bio-availability (if information available)**	**(Intermediate) endpoints**	**Notable results**
			Intervention group (n)	**Control group (n)**			
Kojuri et al. 2007, Iran; 6 wk single-blind RCT [Kojuri, 2007 #75]	- Enteric-coated garlic powder tablet (~ 4g garlic, 1 mg allicin; 2/d) + NCEP type II diet OR - Anethum tablet (650 mg; 2/d) + NCEP type II diet	Hyperlipidemic subjects (TC ≥ 200 mg/dl and/or LDL-C ≥ 100 mg/dl after 10 hours of fasting) with coronary artery disease (mean age 55.8 y; both sexes)	- Enteric-coated garlic powder tablet (50) - Anethum tablet (50)	Placebo (50)		TC, LDL-C, HDL-C, TG	Garlic may play an important in therapy of hypercholesterole Garlic tablet has signifi favorable effect on TC, LDL-C, HDL-C
Gardner et al. 2007, US; 6 mo, parallel-design RCT [Gardner, 2001 #77]	- Raw garlic (~ 4g garlic clove; 6d/wk) OR - Powdered garlic supplement (~ 4g garlic clove; 6d/wk) OR - Aged garlic extract supplement (~ 4g garlic clove; 6d/wk)	Adults with moderate hypercholesterolemia (LDL-C conc. of 3.36-4.91 mmol/L) (aged 49-52; both sexes)	- Raw garlic (49) - Powdered garlic supplement (47) - Aged garlic extract supplement (48)	Placebo (48)		- LDL-C concentrations - Fasting plasma lipid concentrations (TC, HDL-C, LDL/LDL ratio, TG)	Garlic supplementation or die garlic in reasonable doses unlikely to produce lipid bene No statistically significant effec LDL-C conc. or other plasma conc.
Doorn et al. 2006, Netherlands; 12 wk, RCT [van Doorn, 2006 #115]	Garlic powder (2.1g/d)	Subjects with risk factors for CVD (BMI > 24.5, aged 40-75 y who smoked >10 cigarettes/d) (both sexes)	- Garlic powder (28)	- Placebo (26) - Atorvastatin (30)		- Inflammatory markers (CRP, TNF-alpha, fibrinogen) - Endothelial function markers (von Willebrand factor, s-ICAM, s-VCAM) - Lipid profile (TC, HDL-C, LDL-C, TG)	Garlic powder has no benef effects on atherogenesis: significant effect on inflamma biomarkers, endothelial funct or lipid profile in normolipide subjects with risk factors for C\

Zhang et al. 2006, China; 2 x 2 x 2 and 2 x 2 factorial RCT [Zhang, 2006 #79]	2-wk twice-daily treatment with 1 g amoxicillin and 20mg omeprazole and supplementation throughout the study with 1) 2 capsules twice daily, each containing 200mg aged garlic extract and 1 mg steam-distilled garlic oil, or 2) twice-daily micronutrient capsules containing 250mg vitamin C, 100 IU vitamin E, and 37.5mg selenium	Healthy, rural, Chinese population (aged 35-64 y; both sexes)	2285 HP-seropositive subjects entered 2x2x2 factorial trial of antibiotics, vitamins, and garlic	1126 HP-seronegative subjects entered 2x2 factorial trial of vitamins and garlic		TC, HDL-C, LDL-C	In this rural Chinese population with low meat intake and moderate cholesterol concentrations, long-term garlic supplementation had no effect on lipid profiles
Tanaka et al. 2006, Japan; 12 mo preliminary RCT [Tanaka, 2006 #80]	Aged Garlic Extract (AGE) (2.4 mL/d)	Patients with colorectal adenomas-precancerous lesions of the large bowel	AGE 2.4 mL/d (19)	AGE 0.16 mL/d (18)		Size and number of colon adenomas	The results suggest that AGE suppresses progression of colorectal adenomas in humans: Significant suppression of both the size and number of colon adenomas
Ishikawa et al. 2006, Japan; 6 mo, RCT [Ishikawa, 2006 #81]	Aged Garlic Extract (AGE) (5g/d)	Patients with inoperable colorectal, liver, or pancreatic cancer (aged ≥ 20 y)	AGE (18)	Placebo (23)		Quality of Life, natural-killer (NK) cell activity, salivary cortisol level	Administering AGE to patients with advanced cancer of the digestive system improved NK cell activity: Significant increase in both the number of NK cells and the NK cell in the AGE group
Williams et al. 2005, New Zealand; 6 wk, cross-over RCT [Williams, 2005 #82]	Aged 'Kyolic' Garlic Extract (AGE) (2.4g/d, with 2wk wash-out period)	Men with proven coronary artery disease (aged 45-70 y)	AGE (15)			- Oxidant stress (plasma oxidized LDL-C, peroxides) - Systemic inflammation (plasma CRP, IL-6) - Endothelial activation (VCAM-1) - Lipid profile (TC, HDL-C, LDL-C, TG) - Flow mediated dilation (FMD) and glyceryl trinitrate induced vasodilatation (GTN) of brachial artery	Short-term treatment with AGE may improve impaired endothelial function in men with CAD treated with aspirin and a statin: During AGE supplementation, FMD increased significantly from the baseline and mainly in men with lower baseline FMD. Levels of FMD at the end of AGE treatment were significantly higher compared with the corresponding levels at the end of placebo treatment when the variation in baseline body weight was taken into account. Markers of oxidant stress, systemic inflammation and endothelial activation did not change significantly

Tanamai et al. 2004, Thailand; 9 mo cross-over RCT [Tanamai, 2004 #76]	Enteric-coated garlic extract tablets (5000µg/tablet, 1.5% of allicin)	Subjects with high serum lipid level (TC > 250 gm/dl) (aged ≥ 35 y)	Trial group (45): 3 mo garlic tablets, followed by 3 mo placebo tablets and 3 mo of tablets discontinuity the volunteers in the control group started with three months of placebo followed by three months of garlic tablets and ended up with three months of tablets discontinuity	Control group (55): 3 mo placebo tablets, followed by 3 mo garlic tablets and 3 mo of tablets discontinuity		- Lipid profile (TC, HDL-C, LDL-C, TG) - Blood sugar, liver, kidney functions and complete blood counts	Garlic tablets used in this study did not contribute to either lowering serum cholesterol and TG levels or increasing HDL levels: No significant differences between the two groups at the end of 3 mo or 6 mo of the study
Turner et al. 2004, Denmark; 12 wk RCT [Turner, 2004 #83]	Dried garlic powder tablets (10.8 mg alliin (3-(2-propenylsulfinyl)-L-alanine)/d ~ 3 garlic cloves/d)	Healthy, normo-lipidaemic subjects	Garlic tablets (31)	Placebo (32)		- Blood lipids (TC, LDL-C, HDL-C. TG), - Blood pressure - Arterial stiffness	Garlic powder tablets have no clinically relevant lipid-lowering and blood pressure -lowering effects in middle-aged, normo-lipidaemic individuals: No significant differences between the garlic and placebo groups were detected for any of the outcome measures
Peleg et al. 2003, Israel; 16 wk RCT [Peleg, 2003 #84]	Garlic powder tablets (22.4 mg/d alliin)	Patients with primary hypercholesterolemia and no evidence of cardiovascular disease (aged 18-80 y)	Garlic tablets (13)	Placebo (20)		- Lipid profile - Psychopathologic parameters	Short-term garlic therapy in adults with mild to moderate hypercholesterolemia does not affect either lipid levels or various psychopathologic parameters: No significant changes lipid profile, or in the psychopathologic parameters evaluated

Appendix VI

Phytochemical: Ellagic acid **Database(s):** Medline **Search:** "Ellagic acid"[MeSH] **Limits:** English, Clinical Trial, Randomized Controlled Trial							
Author, year, location; intervention length and research design	**Intervention (dose, chemical form)**	**Subjects (age, gender, special properties/disease)**	**Study group**		**Bioavailability (if information available)**	**(Intermediate of) endpoints**	**Notable results**
			Intervention group (n)	**Control group (n)**			
1) Falsaperla et al. 2005, Italy; 6 wk single-blind RCT (Falsaperla, Morgia et al. 2005)	daily dose of 180 mg (60 g/every 8 h) active principle and administered orally before meals with water (200– 250 ml) throughout the chemotherapy cycles and during the period between cycles. Ellagic acid was HPLC extracted from Punica granatum seeds Each capsule contains 30 mg of active principle in the form of ellagic tannis obtained from 75 mg concentrated extract of standardized seeds at 40%.	consecutive patients (median age 66.5 years, range 58–71) with hormone refractory prostate cancer, chemotherapy-naïve, were recruited. Inclusion criteria: Karnofsky performance status score ≥70; pain visual analogic-numeric scale ≤6; pain index (analgesics/day) ≤5; normal bone marrow, renal and liver functions	n=24 chemotherapy treatment with vinorelbine (25 mg/mq, weekly, for 6 weeks) and estramustine (280 mg, thrice daily, for 42 days) And ellagic acid capsules	n=24 chemotherapy treatment with vinorelbine (25 mg/mq, weekly, for 6 weeks) and estramustine (280 mg, thrice daily, for 42 days)		Chemotherapy induced toxicity (e.g. neutropenia, anemia, nausea, anorexia), objective response (OR), individual clinical response (ICR) and Biochemical response (BR) with use of o.a. the following parameters: PVA-NS, PI (Pain Index), KPSS and PSA levels.	A reduction in systemic toxicity, statistically significant for neutropenia, associated with better results in term of OR, ICR, and BR was observed in the intervention group compared with the control group. No significant difference in overall survival and progression-free survival was detected between both groups, though positive trend were observed.

Appendix VII

Phytochemical: Proanthocyanidins
Database(s): Medline
Search: "Proanthocyanidins"[MeSH]]
Limits: English, Clinical Trial, Randomized Controlled Trial, Humans

Author, year, location; intervention length and research design	Intervention (dose, chemical form)	Subjects (age, gender, special properties/disease)	Study group		Bioavailability (if information available)	(Intermediate of) endpoints	Notable results
			Intervention group (n)	Control group (n)			
Brooker et al.; 2006, UK; 6 month intervention, endpoints at 12 months; intervention with placebogroup (Brooker, Martin et al. 2006)	100 mg IH636 grape seed proanthocyanidin extract (GSPE) three times a day, orally, for 6 moths	Female; eligibility criteria: palpable breast induration (tissue hardness) due to radiotherapy for early breast cancer delivered at least 24 months prior to trial entry, freedom from cancer recurrence, availability for follow-up	n=44 100 mg GSPE three times a day, orally, for 6 months	n=22 corresponding placebo capsules	No significant differences between treatment groups in antioxidant effect in serum and urine. However, this does not exclude the absorption of biologically active Metabolites.	Primary endpoint: change in area (cm2) of skin markings outlining induration 12 months after randomisation measured by planimetry coded as a binary response variable with response defined as a reduction in area of at least 50%. Secondary endpoints: (i) external grading of hardness, (ii) photographic breast appearance, (iii) patient self-assessment of breast hardness, pain and tenderness.	No efficacy of orally-adminstered GSPE in patients with breast induration following radiotherapy for breast cancer was shown
Howell et al.; 2005, USA; 1 day intervention, 3 day wash-out , repeated for 5 different products; cross-over trial (Howell, Reed et al. 2005)	240 mL of commercially available cranberry juice cocktail (contains A-linked proanthocyanidins) , single dose	six healthy volunteers (4 women and 2 men) between the ages of 25 and 45	n=6 240 mL commercially available cranberry juice, single serving	n=6 4 times a food product containing B-linked proanthocyanidins: 240 mL purple grape juice / 240 mL apple juice/ the aqueous extract from 2 g of brewed green tea / 40 g of chocolate, all single servings		Urinary bacterial (E.coli) anti-adhesion activity	Bacterial anti-adhesion activity was only detected in the urines of those volunteers that consumed a single serving of the cranberry juice cocktail

Yamakoshi et al,; 2004, Japan; 12 months; open design study (Yamakoshi, Sano et al. 2004)	67 mg of grape seed extract (GSE) containing 81.0% proanthocyanidins. orally administered three times a day for, and for 5 months between the first day of March 2002 and the end of July 2002. Extracts were prepared from grape seeds (*Vitis vinifera* L.).	Non-pregnant Japanese woman with chloasma.	n=12 (6 months between 01-08-2001 and 31-01-2002) 67 mg of GSE, three times a day, orally, for 6 months n=11 (5 months between 01-03-2002 and 31-07-2002) GSE, three times a day, orally, for 5 months			Clinical observation, L*-values (indicating the lightness of the skin color), melanine index and size measurements of chloasma.	GSE is effective in reducing the hyperpigmentation of women with chloasma. The beneficial effects of GSE were maximally achieved after 6 months and these was no further improvement after this period.
Vigna et al.; 2003, Italy; 4 wk intervention, 3wk wash-out, 4 wk intervention; randomized, double-blind, crossover study (Vigna, Costantini et al. 2003)	A standardized grape seed extract (Leucoselect-Phytosome (LP)) containing 75 mg grape seed extract, 192 mg soy phosphatidylcholine, starch, silicon dioxide, and other excipients. 2 capsules were given twice daily at the end of main meals (lunch and dinner), for a total equivalent of 300 mg grape procyanidin extracts,	Healthy men, aged 50 or more, smoking >10 cigarettes a day	n=21 capsules with grape seed extract	n=21 placebo capsules		Total cholesterol (TC), tryglicerides (TG), LDL- and HDL-cholesterol LDL-C;HDL-C), FPLP, Lag Phase, Propagation Rate, and TBARS.	No significant modification of TC, TG, (HDL-C) and LDL-C during LP treatment. TBARS were significantly reduced and the Lag-phase was significantly prolonged.

Appendix VIII

Phytochemical: Anthocyanins
Database(s): Medline
Search: "Anthocyanins"[MeSH]]
Limits: English, Clinical Trial, Randomized Controlled Trial, Humans

Author, year, location; intervention length and research design	**Intervention (dose, chemical form)**	**Subjects (age, gender, special properties/disease)**	**Study group**		**Bioavailability (if information available)**	**(Intermediate of) endpoints**	**Notable results**
			Intervention group (n)	**Control group (n)**			
Weisel; 2006, Germany. 9 wk (2w run-in; 5 wks intervention; 2 wks wash-out) intervention with placebogroup (Weisel, Baum et al.)	700 mL Mixed fruit juice, produced from red grape juice (57%), blackberry juice (18%), sour cherry juice (9%), black currant juice (9%), and chokeberry juice (7%). Juice was consumed daily in 3 equal portions	Male healthy non-smoking probands were recruited from the University of Kaiserslautern. Exclusion criteria were smoking, major obesity (BMI >30), chronic diseases and use of medication.	n=18 700 mL/day of the described juice	n=9 (not at same time as intervention) 700 mL/day of control juice (= juice similar as intervention juice, but without chokeberries and removal of pholic fraction)		Biomarkers of oxidative cell damage (DNA damage, MDA, glutathione status) and selected modulation of cell response (total glutathione, NF-κB-DNA binding activity) were determined.	Oxidative DNA-damage and an increase in reduced glutathione and glutathione status showed a significant decrease. MDA, DNA-binding activity of NF-κB and were not significantly altered.
Enomoto et al.; 2005, USA. Radiation therapy period + 2 months; RCT (Enomoto, Johnson et al. 2005)	RayGel (an aqueous-based formulation containing reduced glutathione and anthocyanins), which was applied the participants' breasts 1 to 3 hours prior to radiation therapy.	Patients undergoing whole breast external-beam irradiation All had breast-conserving surgery. Women with a history of mastectomy or previous chest wall irradiation were excluded. All received standard whole breast treatment with tangential beam irradiation using computed tomography–based treatment.	n=15 Raygel	n=15 Placebo gel		Photographs were taken every 2 weeks during therapy and at 2 months following completion of the course of radiation. Severity score was assigned after evaluation of the photographs. . Scoring was based on a modification of the Radiation Therapy Oncology Group (RTOG) acute radiation morbidity scoring system.	A trend was seen toward improvement in the RayGel group. This trend was however not significant.

Lee et al.; 2005, Korea. 4 wk randomized, double-blind, placebo-controlled trial. (Lee, Lee et al. 2005)	Purified high-dose anthocyanoside oligomers, given in the form of the clinical investigation product Eyezone (100 mg tablets consisting of 85% anthocyanoside oligomers) 1 tablet, twice daily for 4 weeks.	people with poor nocturnal vision and symptoms of asthenopia Inclusion criteria: a refractive error between 21·00 and 28·00 diopters in both eyes, decreased night vision, and asthenopia classified as severe based upon results of a structured questionnaire.	n=30 Eyezone tablets	n=30 Placebo		subjective symptoms (determined by questionnaire) and objective contrast sensitivity	The anthocyanoside group had a significantly greater improvement than the placebo group on subjective symptoms. The mean contrast sensitivity change in the anthocyanoside group was significantly improved compared to the placebo group.
Matsumoto et al.; 2005, Japan. 2 (2 day and 2 week) double-blind, placebo-controlled, crossover studies (Matsumoto, Takenami et al. 2005)	blackcurrant anthocyanin (BCA) concentrate capsules prepared from a commercially available blackcurrant juice, containing 10.83% anthocyanins.	Study 1(resting circulation): Healthy males Study 2 (typing work): Healthy subjects All subjects did office work for at least 3 years, working ca. 3-6 hours/day on a visual display terminal (VDT)	Study 1: n=9 BCA- concentrate capsules (17 mg (kg body weight)-1), single dose Study 2: n= 11 capsules of BCA (7.7 mg (kg body weight)-1 daily for 2 weeks.	Study 1: n=9 placebo capsules (isoenergetic sugar), single dose Study 2: n= 11 placebo (isoenergetic sugar) daily for 2 weeks.		Study 1: left forearm blood flow (FBF) following venous occlusion and muscle oxygen consumption following arterial occlusion prior to and hourly for 4 h after ingestion of BCA. Study 2: Total hemoglobin and oxygenated hemoglobin (oxy-Hb) and myoelectric signals in the right trapezius muscle (EMG) were measured during workload. The viscoelasticity of the trapezius muscle was determined before and after typing workload.	Study 1: FBF increased significantly 2 h after BCA ingestion and tended to increase for a further 3 h after ingestion. There was no significant difference in muscle oxygen consumption between BCA and placebo intake at any time point. Study 2: BCA intake prevented the decrease in oxy-Hb significantly. There was a non significant tendency to alleviate the increase in root mean square (RMS) of the EMG during the typing workload, and also muscle stiffness after the workload. There was no improvement in typing performance with BCA intake

Moller et al,; 2004, Finland; 3-wk controlled parallel intervention study (Moller, Loft et al. 2004)	Intervention group 1: blackcurrant juice containing 600 mg/l anthocyanins and 210 mg/l Vitamin C. Intervention group 2: anthocyanin drink 600 mg/l anthocyanins but no Vitamin C . The daily dose of the drinks was based on the bodyweight (either 475, 630, 790, or 1000 ml/d). The drinks were ingested during three daily meals.	Subjects recruited from the University of Helsinki Viikki campus and the neighboring residential area. Exclusion criteria were current diseases (including metabolic diseases), large overweight (BMI > 30 kg/m2), special dietary require	n=18 Blackcurrant juice n=20 anthocyanin drink	n=19 control drink		Oxidative DNA damage in mononuclear blood cells and plasma was measured by determining Strand breaks, Endo III sites, and Fpg sites.	No decrease on steady state levels of oxidative DNA damage were found.
Muth et al,; 2000 10 wk (3 wk intervention, 1mont wash-out, 3 wk intervention) randomized, controlled, double-blind, cross-over study (Muth, Laurent et al. 2000)	capsules containing 160 mg bilberry extract of which 25 percent were anthocyanosides, 1 capsule three times daily.	males recruited from personnel working at Naval Air Station Pensacola, Florida. All subjects had visual acuity correctable to 20/20 or better.	n=15 bilberry extract capsules, 1 capsule three times daily	n=15 placebo capsules, 1 capsule three times daily		night visual acuity (VA) and contrast sensitivity (CS)	No effect on VA and CS were was found.
Nakaishi et al,; 2000, Japan. 2 (max. 5 h) double-blind, placebo-controlled, crossover studies (Nakaishi, Matsumoto et al. 2000)	Powdered black currant anthocyanoside (BCA) concentrate was prepared from a commercially available black currant juice. The concentrate contained 9.2-percent BCA.	Dark Adaptation Study (Study 1): Healthy subjects, showing no pathological ocular signs. Transient Refractive Alteration Study (study 2): Healthy subjects, confirmed to be free of any ocular diseases, refractive errors (high myopia more than 4 diopter, hyperopia more than 1 diopter, or astigmatism of which the strongest curvature was more than 1.5 diopter), or presbyopia at the time of enrollment	Study 1 n=12 three groups given doses of BCA concentrate (540, 270, and 135 mg/subject, corresponding to 50, 25, and 12.5 mg BCA/ subject). Each dose of BCA was orally ingested as six capsules. Study 2: n=11 (200 mL) juice containing BCA concentrate (540 mg/ subject, corresponding to 50 mg of BCA/subject)	Study 1 n=12 placebo capsules Study 2: n=11 Placebo juice		Study 1: Dark adaptation threshold Study 2: spherical (R) and cylindrical (C) refraction of the dominant eye, M_{cff} value (flicker value), and subjective fatigue symptoms	Study 1: Intake of BCA at three dose levels (12.5-, 20-, and 50 mg/subject) appeared to bring about dose-dependent lowering of the dark adaptation threshold (not significant). Study 2: Subjective asthenopia symptoms reagarding the eye and lower back improved significantly. Other endpoints were not significant.

Appendix IX

Study type: Human Intervention Study
Subject category: Healthy
Database(s): Medline
Search: ("lycopene "[Substance Name]) OR ("Lycopersicon esculentum"[Mesh])
Limits: published in the last 2 years, Humans, Clinical Trial, Randomized Controlled Trial, English

Author, year, location; intervention length and research design	**Intervention (dose, chemical form)**	**Subjects (age, gender, special properties/disease)**	**Study group**		**Bioavailability (if information available)**	**(Intermediate of) endpoints**	**Notable results**
			Intervention group (n)	**Control group (n)**			
Cancer							
Riso et al. 2006, Italy; 26d, cross-over RCT [Riso, 2006 #1]	250 ml/d of a tomato drink (Lyc-o-Mato) (5.7mg/d lycopene)	Healthy subjects (mean age 26.0 ± 2.9 y, both sexes)	Lyc-o-Mato → Placebo drink (20 → 20)	Placebo drink → Lyc-o-Mato (20 → 20)	Plasma lycopene levels increased significantly	IGF-1	No significant modulation of IGF-1 levels following the intake of low amounts of lycopene and other carotenoids provided by a tomato drink. However, a significant reduction of IGF-1 serum was observed in subjects with the highest plasma lycopene response but also IGF-1 levels following the tomato drink intake. Further exploration is necessary
Riso et al. 2006, Italy; 26d, cross-over RCT [Riso, 2006 #23]	250 ml/d of a tomato drink (Lyc-o-Mato) (5.7mg/d lycopene)	Healthy subjects (mean age 25.7 ± 2.1f or group 1 and 25.9 ± 3.4 years for group 2, both sexes)	Group 1: Lyc-o-Mato → Placebo drink (26 → 26)	Group 2: Placebo drink → Lyc-o-Mato (26 → 26)		Cytokine determination, assessment of DNA	TNF-α production by whole blood was 34.4% lower after 26 days of drink consumption, whereas the other parameters were not significantly modified by the treatment; modest effects of the regular intake of a tomato drink, were found on the production of inflammatory mediators, such as TNF

Zhao et al. 2006, US; 56d, RCT [Zhao, 2006 #34]	12 mg/d Synthetic lycopene , or 4 mg/d synthetic lycopene as part of mixed carotenoids	Healthy, nonsmoking, postmenopausal (mean age 60 ± 2y, women)	- Only lycopene (8) - Lycopene as part of mixed carotenoids (8)	Placebo (8)	Lycopene plasma levels increased at 56d vs baseline in supplemented group	DNA damage in PBMCs	Carotenoid supplementations decreases DNA damage: In both intervention groups endogenous DNA damage decreased significantly vs. baseline and placebo
Porrini et al. 2005, Italy; 26d, cross-over RCT[Porrini, 2005 #28]	250 ml/d beverage (Lyc-o-Mato) containing a natural tomato extract (5.7mg/d lycopene)	Healthy subjects (mean age 25.8 ± 2.8 y, BMI 21.3 ± 1.7 kg/m2)	Lyc-o-Mato → Placebo drink (26 → 26)	Placebo drink → Lyc-o-Mato (26 → 26)	Plasma lycopene levels increased significantly at 26 days versus baseline	DNA damage in lymphocytes	This study supports the fact that a low intake of carotenoids from tomato products improves cell antioxidant protection: 42% decrease (significant) in DNA damage in Lymphocytes
Cardiovascular Disease							
Misra, et al. 2006, India; 6mo RCT (Misra, Mangi et al. 2006)	Lycopene supplementation LycoRed (2000 µg lycopene)	Healthy postmenopausal women	LycoRed (20)	Hormone Replacement Therapy HRT (21)		Serum lipid profile, marker of lipid peroxidation (malondialdehyde), level of endogenous antioxidant (glutathione)	LycoRed can be used as an alternative to HRT to reduce the risk of atherosclerosis in postmenopausal women: significant effects in both groups
Bub et al. 2005, Germany; 2wk, cross-over randomized study [Bub, 2005 #29]	330 ml/d of tomato juice (37 mg/d lycopene)	Healthy subjects with different PON1-192 genotypes (mean age 29 ± 2y, men)	(22)	-	Plasma lycopene levels increased at 2 wk vs baseline	PON1-192 genotype, *ex vivo* LDL oxidation (lag time), plasma malondialdehyd, PON1 activity	Tomato juice intake might contribute to CVD risk reduction: Decreased lipid peroxidation in volunteers carrying the R-allele of the PON1-192 genotype; significant decreased plasma malondialdehyde in QR/RR subjects

Study type: Human Intervention Study
Subject category: Not healthy
Database(s): PubMed
Search: ("lycopene "[Substance Name]) OR ("Lycopersicon esculentum"[Mesh])
Limits: published in the last 2 years, Humans, Clinical Trial, Randomized Controlled Trial, English

Author, year, location; intervention length and research design	Intervention (dose, chemical form)	Subjects (age, gender, special properties/disease)	Study group		Bioavailability (if information available)	(Intermediate of) endpoints	Notable results
			Intervention group (n)	Control group (n)			
Prostate cancer							
Jatoi et al. 2007, USA; 4 mo, Intervention study without control group(Jatoi, Burch et al. 2007)	Tomato-based supplement (15mg ycopene, twice daily). This supplement consisted of either tomato paste or tomato juice.	Patients with androgen-independent prostate cancer (aged 55-80 y, only males)	46	_		50% decline in serum PSA level	Lycopene, as prescribed in this study, did not appear effective for androgen-independent prostate cancer: only 1 patient had a 50% or greater decrease in serum PSA
Edinger et al. 2006, Brazil; 10wk, Intervention study without control group(Edinger and Koff 2006)	50 g/d (3 soup spoons) of tomato paste (≈ 13 mg/d lycopene)	Patients with benign prostate hyperplasia (aged 45-75 y, only male, PSA levels of 4-10 ng/mL)	43	-		PSA levels	Significant reduction of mean plasma PSA levels in patients with benign prostate hyperplasia
Cardiovascular Disease							
Bose et al. 2007, India; 60d, RCT (Bose and Agrawal 2007)	200 g/d of ripe, cooked tomatoes for 60 days (≈ at least 25 mg/day Lycopene)	Patients with CHD and an aged-matched control group (aged 35-55 y, both sexes)	30	50		- Oxidative stress biomarkers: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and reduced glutathione (GSH) - Lipid peroxidation rate: thiobarbituric acid reactive substances (TBARS) - Lipid profile: TC, TG, HDL, LDL, VLDL	Tomato lycopene may have considerable therapeutic potential as an antioxidant but may not be used as hypolipidaemic agent in CHD: Significant improvement in the levels of serum enzymes involved in antioxidant activities and decreased lipid peroxidation rate but no significant changes in lipid profile

Engelhard, 2006, Israel; 8wk, single-blind RCT[Engelhard, 2006 #24]	250 mg/d of an encapsulated tomato extract, Lyc-O-Mato (15/d mg lycopene)	Patients with grade-1 Hypertension (HP) (aged 30-70 y, both sexes)	4-week placebo period, then an 8-week treatment period with tomato extract, and a 4-week control period with placebo (31)			Systolic and diastolic blood pressure in grade-1 HT, serum lipoproteins, plasma homocysteine, oxidative stress markers	A short-term treatment with tomato extract can reduce blood pressure in patients with grade-1 HT, long term effects still need to investigated: Systolic blood pressure and diastolic blood pressure decreased, no changes in blood pressure were demonstrated during placebo periods; Thiobarbituric acid–reactive substances, a lipid peroxidation products marker, decreased; no significant changes were found in lipid parameters.
Other human diseases							
Kumar et al. 2007, India; 2 mo, RCT(Kumar, Bagewadi et al. 2007)	Lycopene capsules (16 mg/d of lycopene) with or without intralesional steroid injections	Patients with oral submucous fibrosis (median age 28 y, only male)	- Group A: Lycopene capsules only (21) - Group B: Lycopene capsules + steroid injections (19)	Group C (18)		Mouth opening, visual inspection, palpatory findings, burning sensation	Lycopene can and should be used as a first line of therapy in the initial management of oral submucous fibrosis: Mouth-opening values for the patients showed an average increase of 3.4 mm, 4.6 mm, and 0.0 mm for patients in groups A, B, and C, respectively. These values were statistically significant.