



Tomato, Lycopene and Health Workshop

Avignon, Thursday 22nd October 2009





Program

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8.30 a.m. Arrival and registration

9 a.m. – 12.40 p.m. Morning session

- Welcome and opening presentations by **Catherine Caris-Veyrat, Sophie Colvine** and **Volker Böhm, Coordinator of the LYCOCARD project** 10'
- Moderator : Volker Böhm*
- George Britton** - Lycopene: the autobiography 30' + 10'
- Kati Fröhlich, LYCOCARD** - Isolation, structural elucidation and stability of geometrical isomers of lycopene 10' + 5'
- James Giovannoni** - Molecular strategies toward identifying regulators of nutritional pathways in tomato 30' + 10'
- Céline Chanforan** - Identification and quantification of carotenoid-derived products in an emulsion modelling the processing of tomato paste into tomato sauce 10' + 5'

Coffee/Tea break (10.45-11.15 a.m.) and Poster Session

- Moderator : Patrick Borel*
- Javier Garcia, LYCOCARD** - Stability of bioactive compounds during storage of tomato juice 10' + 5'
- Wilhelm Stahl** - Lycopene : in-vitro and in-vivo studies 30' + 10'
- Daniel. L. Graham, LYCOCARD** - Cigarette smoking and human plasma lycopene depletion. 10' + 5'

Buffet lunch (12.45-2.00 p.m.)

2.00 – 5.45 p.m. Afternoon session

Moderator : **Catherine Caris-Veyrat**

John W. Erdman - What do we know about the metabolism of lycopene ? 30' + 10'

Eric Reynaud, LYCOCARD - Organic synthesis of apo-lycopenoids and their antioxidant properties in biomimetic system 10' + 5'

Yoav Sharoni - Mechanisms for cancer prevention by carotenoids and their derivatives 30' + 10'

Ralph Rühl, LYCOCARD - Lycopene is a potent activator of RAR-mediated response pathways in an RARE animal reporter mouse model 10' + 5'

Break 3.50 – 4.05 p.m. and Poster Session

Moderator : **Ralph Rühl**

Kazim Sahin – Lycopene and chemotherapy toxicity 20' + 5'

Rossella Simone, LYCOCARD - Preventive role of lycopene on oxysterol-induced pro-inflammatory cytokine cascade in human macrophages 10' + 5'

Gwen Young - Processed tomatoes on vasodilatation and C-reactive protein (hsCRP) in overweight and obese men and women 30' + 10'

Janine Kalkowski, LYCOCARD - Lycopene and endothelial function 10' + 5'

Catherine Caris-Veyrat, Sophie Colvine and Volker Böhm
Concluding remarks 5'



Oral Presentations Abstracts

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LYCOPENE: THE AUTOBIOGRAPHY

George Britton

The University of Liverpool, School of Biological Sciences, Crown Street, Liverpool L69 7ZB, UK. (e-mail: george.britton19@gmail.com)

Much is said and written about lycopene and how it may influence human health. Other presentations will report experimental studies of such effects of lycopene in relation to, for example, metabolism, cancer and cardiovascular disease.

Many fundamental and challenging questions remain, however. Is lycopene an antioxidant or a pro-oxidant? What is the importance of *cis* (*Z*) isomers and isomerisation? How does lycopene break down, and do products of this breakdown have biological activity?

Lycopene looks like a simple carotenoid, but it does not have a simple character. Rather, it has a split/multiple personality. A key factor in this is the strong tendency to form aggregates, and this significantly affects not only solubility and light absorption but a range of other important features that underlie the biological properties of lycopene. This first talk will tell lycopene's own story, covering aspects such as structure, aggregation, isomerisation, solubility, stability, formation of radicals, redox properties, physical quenching and chemical reactivity, to provide a background for understanding the properties and behaviour of lycopene *in vivo* and for evaluating reported biological effects.

Isolation, structural elucidation and stability of geometrical isomers of lycopene

Kati Fröhlich

Friedrich-Schiller-University Jena, Institute of Nutrition, Bioactive Plant Products, Dornburger Str. 25-29, 07743 Jena, Germany, kati.froehlich@uni-jena.de

Lycopene ($C_{40}H_{56}$) is a symmetric, unsaturated hydrocarbon with 11 linearly arranged conjugated double bonds. Theoretically, lycopene has 2048 (10^{11}) different stereoisomers, but some isomers are sterically inhibited. The number of possible stereoisomers can be calculated depending on the symmetry of the chains and odd/even number of effective double bonds [1]. The 72 possible isomers of lycopene include (*all-E*)-lycopene, 4 mono-(*Z*)-, 12 di-(*Z*)-, 19 tri-(*Z*)-, 19 tetra-(*Z*)-, 12 penta-(*Z*)-, 4 hexa-(*Z*)-, and 1 hepta-(*Z*)-lycopene [2]. Lycopene from tomatoes and tomato-based food products exists predominantly in (*all-E*)-configuration, thermodynamically the most stable. In contrast, various (*Z*)-isomers account for over 50% of blood lycopene and for over 75% of tissue lycopene. However, in most investigations on lycopene, the different (*Z*)-isomers are only tentatively identified by using the UV-Vis data or/and their HPLC retention behavior compared to published separations. Thus, the objective was to isolate prominent geometrical lycopene (*Z*)-isomers as well as to unambiguously identify these isomers by using different spectroscopic methods.

A saturated solution of (*all-E*)-lycopene in cyclohexan/toluene (4+1, v/v) was photo-isomerized according to the method of Zechmeister [1] using iodine and UV light or was heat-isomerized. Different isomers of lycopene were isolated on a preparative C_{30} (300 x 10.0 mm, 5 μ m) column (YMC Europe, Schermbeck, Germany). Mixtures of methanol and methyl tert. butylether were used as mobile phase.

Five geometrical isomers of lycopene were isolated from a photoisomerized mixture and unambiguously characterized as (*5Z,9Z*)-, (*9Z*)-, (*5Z,9Z*)-, (*all-E*)-, and (*5Z*)-lycopene by UV-vis spectroscopy, mass spectroscopy (MS), and nuclear magnetic resonance (NMR) spectroscopy [3]. Recently, another isomer which is also found in food as well as in humans and animals was isolated to characterize the exact structure of the isomer by MS and NMR. Experiments to characterize this isomer via UV/Vis data (ratio of absorption intensity at the near-UV maximum ("cis-Peak") to absorption intensity at the main absorption maximum) showed that this isomer is either (*13Z*)- or (*15Z*)-lycopene. But it was not possible to characterize the isomer by using these calculated ratios from more than 35 UV/Vis spectra due to high variations in ratio (0.41-0.66) [2]. This isomer was unambiguously identified as (*13Z*)-lycopene by using different NMR data (1H NMR, 2D NMR including gCOSY, TOCSY; TROESY). ROESY-correlation between methyl group Me-20 (δ 1.99 ppm) and olefinic proton H14 (δ 6.11 ppm) clearly established the double bond C-13/C-14 as (*Z*)-configuration.

The identification of these isomers of lycopene will allow an identification of the main lycopene isomers in human plasma samples (Figure 1) and food samples by comparison of retention times with standard solutions of isolated lycopene isomers. However, it must be pointed out that the preparation of these isomers is rather difficult and time-consuming as well as that some of these standard solutions are stable for only a couple of days. For example, (*13Z*)-lycopene diluted in mobile phase (MeOH/MTBE, 1+1, v/v), isomerizes in a few hours to other isomers ((*all-E*)-, (*5Z*)-lycopene etc.). Stability tests of isolated lycopene isomers (for 6-8 weeks, crystalline or diluted in different solvent mixtures, different temperatures, with/without argon) showed a better stability of lycopene isomers at lower temperatures, at lower oxygen contents, under argon atmosphere as well as in the darkness.

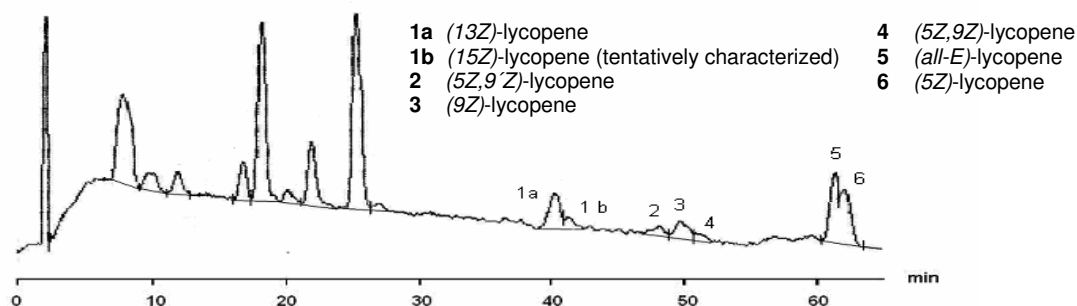


Figure 1: Chromatographic separation of lycopene isomers in a plasma sample using analytical C_{30} column (23 ± 1 °C, 1.3 ml/min, gradient consisting of methanol and MTBE, 450 nm)

References:

- [1] L. Zechmeister, *Chem. Rev.* **34**, 267 (1944)
- [2] K. Fröhlich, PhD thesis, Friedrich-Schiller-University Jena, Germany (2007)
- [3] K. Fröhlich, J. Conrad, A. Schmid, D. E. Breithaupt, V. Böhm, *Int. J. Nutr. Res.* **77**, 369 (2007)

Molecular strategies toward identifying regulators of nutritional pathways in tomato.

James Giovannoni, Ph.D, Lead Investigator

Boyce Thompson Institute for Plant Research, Tower Road, Cornell University campus,
Ithaca, NY 14853 USA. jjg33@cornell.edu

The ripening and development of fleshy fruits is regulated by environmental, hormonal and developmental cues and influences the accumulation of important nutritional metabolites. With the advent of modern genomics approaches and accumulation of genomics-scale data sets we are confronted with challenges in data management, analysis and interpretation and also great opportunities to extract novel meaning from these data if analyzed properly. We have attempted to capture data related to genotype, developmental stage, gene expression and nutritional metabolites and have created a public database to facilitate data management and analysis (Tomato Functional Genomics Database, <http://ted.bti.cornell.edu/>). Emphasis in our group is on the carotenoid, ascorbate and folate biosynthesis pathways during tomato fruit development and ripening. Our goal is to use correlation analysis of gene expression and metabolite levels to identify novel gene candidates associated with ripening and nutrient content. The database and examples of candidate genes and subsequent functional studies will be presented. The database includes data on numerous additional metabolic and phenotypic traits and is meant to assist other researchers in identification of gene candidates associated with pathways, metabolites and traits of interest beyond those included in our studies.

IDENTIFICATION AND QUANTIFICATION OF CAROTENOID-DERIVED PRODUCTS IN AN EMULSION MODELLING THE PROCESSING OF TOMATO PASTE INTO TOMATO SAUCE

Céline Chanforan^{1,2}, Claire Dufour² and Catherine Caris-Veyrat²

¹CTCPA, Site Agroparc, Z.A. de l'aéroport, B.P. 1203, F-84911 Avignon, France

²UMR408 Safety and Quality of Plant Products, INRA, University of Avignon, Domaine St Paul, Site Agroparc, F-84914 Avignon, France
Email : cchanforan@avignon.inra.fr

The nutritional quality of tomato and tomato-derived products is primarily based on their content in antioxidant compounds. In particular, these food products are the main source of (*E*)-lycopene in the western diet, a carotenoid implied in the prevention of degenerative diseases. They also contain (*E*)- β -carotene, a provitaminic A carotenoid. During the industrial preparations of tomato sauce, antioxidants initially present in tomato paste are subjected to high temperature (between 60 and 100 °C) and mixed with vegetable oil. In these conditions, various (photo)chemical reactions may take place leading to the degradation of these micronutrients and the formation of new molecules. The main goals of this work are the identification and the kinetic monitoring of the compounds resulting from the transformation of (*E*)-lycopene and (*E*)- β -carotene in an experimental device modelling tomato sauce preparation.

The emulsion system modelling tomato sauce was achieved by sonicating sunflower oil in an acidic buffer in the presence of vegetable phospholipids. This emulsion contains different markers of the nutritional quality of tomato, i.e. carotenoids: (*E*)-lycopene and (*E*)- β -carotene, phenolic compounds: chlorogenic acid, prunin, rutin and vitamins: ascorbic acid and α -tocopherol. The emulsion was brought to 95 °C and samples were taken out regularly during 30 hours, extracted and analysed using HPLC-DAD-MS. Newly-formed molecules were tentatively identified using UV/Vis and Mass data and by comparison with literature data. Their contents were determined after calibration with available standards.

We describe here the tentative identification of compounds formed from carotenoids. The majority of these products were (*Z*) isomers of (*E*)-lycopene: 4 mono-(*Z*) and 4 di-(*Z*) were tentatively identified and quantified. One oxidation product from lycopene has been observed and assigned to 5,6-epoxy-lycopene. 13-(*Z*)- β -Carotene was the only compound produced from (*E*)- β -Carotene. These products accumulated during the first 8 hours of heating, then declined until a complete disappearance after 28 hours. No further degradation products could be detected. The carotenoid-derived products may have been degraded by cleavage reactions giving volatile compounds which were not trapped in our experimental system.

Finally, degradation products found in the model emulsion were compared with compounds observed in a real tomato sauce product. Most of them were similar, suggesting that our emulsion system may well model tomato sauce processing.

In order to complete the study in model emulsion, a kinetic monitoring of markers of the nutritional quality of tomato sauce is planned on real products, at the laboratory scale.

STABILITY OF BIOACTIVE COMPOUNDS DURING STORAGE OF TOMATO JUICE

Javier Garcia, Javier Casas, Sergio Bravo, Darío Perez, Maria Jesus Periago

Tomato provides an optimal mix of bioactive compounds such as phenolic compounds, folates, ascorbic acid, vitamin E and carotenoids, mainly lycopene, which might be responsible for the proposed health benefits upon tomato consumption. However, technological processing, packaging materials and storage conditions have an impact on the nutritional quality of tomato products, affecting the stability of nutrients to different extents.

We evaluated the stability of antioxidant compounds present in commercially available tomato juices during storage extended for 12 months at 3 different temperatures (8, 22 and 37°C). To further characterize the impact of storage conditions, 2 commonly used packaging materials (tetrapack and glass bottles) were used in order to know whether packaging material affects antioxidants' stability.

Overall, total lycopene, total phenolic compounds and total flavonoids remained substantially stable during storage for 12 months regardless the storage temperature and packaging material used, indicating that tomato juices maintain their nutritional value in terms of antioxidant composition during shelf-life. However ascorbic acid and folates showed to be more labile bioactive compounds and were markedly affected by storage conditions. The hydrophilic TAA paralleled the losses in ascorbic acid content, whereas the lipophilic TAA remained stable throughout the storage trial in agreement with lycopene stability.

Lycopene: in-vitro and in-vivo studies

Wilhelm Stahl, Institute of Biochemistry and Molecular Biology I, University of Duesseldorf, POB 101007, D-40001 Düsseldorf, Germany

Due to its structure lycopene is a carotenoid with distinct chemical and biological activities. It is the colorant of watermelon, rose hip and pink grapefruit but the only major source for lycopene in the human diet are tomatoes. More than 80% of lycopene consumed in the United States is derived from tomato products. Lycopene is a very unpolar non-provitamin A carotenoid found in considerable amounts in human blood and tissues. Processing of food helps to release lycopene from the food matrix, thus improving accessibility of the lipophilic compound for the formation of lipid micelles together with dietary lipids and bile acids. Cooking and food processing enhance the bioavailability of carotenoids; e.g. lycopene uptake is higher after ingestion of processed tomatoes (tomato paste) as compared to fresh tomatoes. Epidemiological studies provide evidence that the consumption of a lycopene-rich diet or selected lycopene-rich food products is correlated with a lower risk for prostate cancer and cardiovascular diseases. A case control study has shown low lycopene plasma levels in patients suffering from congestive heart failure correlating with a decreased ventricular ejection fraction. Patients with atherosclerosis had lower levels of lipophilic antioxidants correlated with elevated biomarkers of oxidative damage and increased intima media thickness. Blood concentrations of lycopene vary with age. In a group of centenarians lycopene levels were about 5-fold lower compared to a group of volunteers aged 60 years and lower. In patients with age related diseases e.g. morbus Alzheimer lower lycopene blood concentrations were found compared to healthy controls.

Based on in-vitro studies it has been speculated that the antioxidant activity of lycopene is at least in part responsible for its beneficial effects in humans. Among carotenoids lycopene is the most efficient singlet oxygen quencher. The interaction of carotenoids with singlet oxygen depends largely on physical quenching, which involves direct energy transfer between both molecules. The energy of singlet molecular oxygen is transferred to the carotenoid molecule to yield ground state oxygen and a triplet excited carotene. Instead of further chemical reactions, the carotenoid returns to ground state dissipating its energy by interaction with the surrounding solvent. Further on lycopene is a radical scavenger interacting synergistically with other carotenoids such as lutein. Upon radical scavenging the molecule decomposes and decomposition products are formed which may also possess biological activities. Lycopene exhibits antioxidant properties in-vivo. After consumption of lycopene-rich diets lycopene levels were increased in blood and the total antioxidant potential in serum was elevated.

Apart from its antioxidant activity other biochemical properties have been attributed to lycopene, which may be relevant in context with its preventing properties. Effects on the proliferation of cancer cells have been associated with the inhibition of cell cycle progression from the G0/G1 to the S phase. The compound also interferes with IGF-1 dependent pathways and induces detoxifying phase II enzymes. There is some evidence that lycopene metabolites or oxidation products play a major role in such signaling pathways.

Studies in cell culture and animals have shown that lycopene prevents UV-induced photo damage. UVB-induced formation of malondialdehyde, a biomarker of lipid peroxidation, was lowered in human fibroblasts in the presence of carotenoids. Based on the levels needed for optimal protection lycopene was a better antioxidant than beta-carotene and lutein. At higher levels all of the investigated carotenoids exhibited prooxidant effects. Human studies on photoprotective effects of lycopene after systemic application are scarce. For most of the investigations lycopene-rich products derived from tomatoes have been used as a source of the carotenoid.

Readout for photoprotection was the prevention of erythema (reddening of the skin) after UV-irradiation. The individual sensitivity towards erythemogenic UV exposure, the MED was determined for each individual prior to the study. For intervention, the volunteers consumed lycopene from different sources for a period of 10-12 wk. At the beginning, during and at the end of the study selected skin areas were irradiated with 1.25-fold the MED using a blue-light solar simulator and skin color was evaluated before and 24 h after irradiation. Skin color was determined by chromametry. Chromametry a-values determine redness of the skin and were used to evaluate the extent of reddening after irradiation. At the time points of UV exposure blood samples were taken and analyzed for lycopene and other carotenoids. Levels of total carotenoids in the skin were measured at the same time points by means of reflection spectroscopy. After ingestion of lycopene or tomato-derived products rich in lycopene, photoprotective effects have been demonstrated. Within 10-12 wk of intervention a decrease in the sensitivity towards UV-induced erythema was observed. Carotenoids including lycopene protect against UV-induced erythema. However, dietary constituents may also influence skin parameters like texture, color, moisture, and other physiological properties. A recent study provided evidence that long-term supplementation with a mixture of antioxidants containing lycopene and beta-carotene, alpha-tocopherol and selenium affects parameters of skin physiology. Applying ultrasound measurements (B-Scan) it was shown that skin density and thickness improved during supplementation. During the study skin surface parameters were determined using the SELS method. Upon supplementation with the antioxidant mixture the SELS parameters scaling and roughness were improved.

It should be noted that endogenous protection associated with the ingestion of dietary carotenoids is not comparable to the topical use of a sunscreen with a high sun protection factor. However, increasing the basal protection and improving skin structure and texture contributes to life-long defense against UV-dependent skin damage.

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Polidori MC, Carrillo JC, Verde PE, Sies H, Siegrist J, Stahl W. (2009) Plasma micronutrient status is improved after a 3-month dietary intervention with 5 daily portions of fruits and vegetables: implications for optimal antioxidant levels. *Nutr J.* 8:10

Cigarette Smoking and Human Plasma Lycopene Depletion.

Daniel. L. Graham¹, Michel Carail², Catherine Caris-Veyrat² and Gordon M. Lowe¹.

¹ School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK.

² UMR408 Securite et Qualite des Produits d'Origine Vegetale, INRA, Universite d'Avignon, France.

Abstract

It is well established that smokers have a higher risk of developing cardiovascular disease and lung cancer. As plasma carotenoid concentrations in smokers are generally lower than in non-smokers, there is currently a debate as to whether this difference is due to modifications in diet, smoking being an appetite suppressant, or whether there is a direct or indirect action of cigarette smoke on carotenoids in the plasma. Recently it was reported that reactive nitrogen species derived from cigarette smoke could diffuse across the lung alveolar cell wall into the plasma. Such species may modify circulating low density lipoprotein (LDL) and in the process reduce circulating carotenoid concentrations. In an effort to address this rational, we have treated lycopene solutions, human plasma and LDL fractions with cigarette smoke and monitored the depletion of all-(E)-lycopene, 5(Z)-lycopene and β -carotene and compared the effects with those of the free radical nitrogen generator Sin-1. These in-vitro experiments have clearly demonstrated that lycopene in plasma and LDL is sensitive to the effects of exposure to cigarette smoke and this may contribute to the low level of lycopene found in smokers.

What do we know about the metabolism of lycopene ?

John W. Erdman Jr., Ph.D.
Department of Food Science and Human Nutrition
University of Illinois
Urbana, Illinois

While decades of research have been devoted to defining the metabolism of beta-carotene for production of vitamin A and other retinoids, efforts to understand the metabolism and identify metabolites from lycopene have only recently been made. Interest in lycopene metabolism has been driven by epidemiological studies and some experimental results suggesting the consumption of lycopene-containing foods or lycopene-containing tomato extracts reduces the risk of cardiovascular disease and some cancers such as prostate cancer. A substantial challenge with these studies has been to differentiate whether lycopene derivatives are the result of metabolism and/or oxidation of lycopene.

The mammalian cleavage enzymes, CMO I and CMO II are the primary carotenoid cleavage enzymes. At high dietary levels, other enzymes such as some of the P450 phase I enzymes have been shown to degrade carotenoids. These enzymes most likely do not play a significant role in metabolism of carotenoids absorbed from foods but may be important following consumption of high dose carotenoid supplements. The primary cleavage of lycopene appears to be via CMO II, the eccentric cleavage enzyme. After initial cleavage of carotenoids by CMO I or II, to yield chain-shortened carotenals, further metabolism to more oxidized forms or further chain-shortening can occur. Essentially nothing is known about downstream metabolism of this carotenoid.

Initial identification of metabolites of lycopene in human blood and breast milk was completed by Khachik and Beecher beginning in the early 1990's. That group suggested that lycopene is oxidized to a 5,6-epoxide which is then further metabolically reduced to 5,6-dihydroxy-5,6-dihydrolycopene and other compounds. Other laboratories have studied *in vitro* production of metabolites of lycopene by exposing pure lycopene to various oxidizing environments. Numerous aldehyde, keto and epoxide chain-shortened lycopene metabolites are formed *in vitro* under these conditions. Most recently, a research group from The Ohio State University fed humans tomato products and identified a variety of apo-lycopenals in blood. These same compounds can be found in mature tomato fruit although the relative concentrations of the apo-lycopenals in tomatoes and human blood differed.

Our laboratory has fed rats diets containing tomato powder or lycopene followed by a single oral dose of 14-C lycopene. We then followed the time-course of lycopene and metabolite accumulation in various tissues. We found a large variety of 14-C labeled more polar metabolites in tissues. In particular, we found 8'-lycopenal and potentially apo-12'-lycopenal in rat liver. In addition, Tufts researchers have found 10'-lyopenol in ferret lung following lycopene feeding.

The creation of mice lacking either or both of the carotenoid cleavage enzymes has provided a valuable tool to study the production of lycopene metabolites. Recent work from our lab with CMO I KO and CMO II KO mice, support the hypothesis that lycopene is not metabolized substantially by CMO I, the central cleavage enzyme. In CMO II KO mice, there was a build-up of lycopene in many mouse tissues suggesting that lycopene is primarily metabolized by CMO II in selected tissues. We also found that CMO I KO mice fed lycopene exhibited significant reduction of hepatic, spleen and thymus lycopene compared to wild type mice fed lycopene. The expression of CMO II is enhanced in CMO I KO mice. The relative

concentration of cis isomers of lycopene increased in KO mice compared to the corresponding wild type mice fed lycopene. Most recently, our laboratory has begun to evaluate the relative tissue expression of CMO I and CMO II in mice fed diets containing either tomato powder or lycopene. Initial results of that work will be presented.

There is considerable emerging evidence that lycopene metabolites have bioactivity which may relate to the “retinoid-like” structures of some of these molecules. As we consider the impact of lycopene on chronic disease development such as cardiovascular disease or cancer, we should be mindful of the relative contributions of the parent molecule, lycopene, and its metabolites. (Supported by NIH grant R01CA125384)

Organic synthesis of apo-lycopenoids and their antioxidant properties in biomimetic system

Eric Reynaud*¹, Pascale Goupy¹, Olivier Dangles¹ and Catherine Caris-Veyrat¹

¹UMR408 Safety and Quality of Plant Products, INRA, University of Avignon, Domaine St Paul, Site Agroparc, F-84914 Avignon, France

Epidemiological studies have shown that regular consumption of tomatoes or derived products have beneficial effects on human health. The carotenoid lycopene together with its metabolites are thought to be involved in the prevention of cardiovascular diseases and cancer. Two mechanisms were suggested: 1- antioxidant property of lycopene particularly in the gastro-intestinal tract / vascular system and 2- cellular effects with the modulation of gene expression by lycopene / lycopene metabolites.

The aim of our study was to obtain molecules which might be endogenous metabolites of lycopene and to examine their antioxidant activities in biomimetic *in vitro* model.

In the LYCOCARD project (IP FP6, "Role of lycopene for the prevention of cardiovascular diseases") we synthesised potential lycopene derived compounds. Four families of molecules were targeted : apo-10'-lycopenoids, apo-12'-lycopénoïds, apo-14'-lycopenoids and appo-11-lycopénoïds. Derivatives with four different ending functions were synthesised: aldehyde, carboxylic acid, alcohol and ester. For the synthesis of apo-lycopenoids, we used organic reactions classically used for the synthesis of carotenoids: *Horner-Wadsworth-Emmons* and *Wittig* coupling together with reduction and oxidation reactions for the functional transformations. Each organic step was optimised in order to obtain the higher possible purity and the only trans-isomer. All molecules were fully characterized using up-to-date analytical techniques: LC/DAD/MS, NMR and HRMS.

The antioxidant activity of the apo-lycopenoids was examined using an experimental model aimed at mimicking an oxidative stress of dietary origin in the gastro-intestinal tract (inhibition of lipidic peroxidation initiated by metmyoglobin). We conclude that for the apo-lycopenoids, a long chain and a terminal COOH group favor the antioxidant activity. Indeed, the terminal group is expected to deeply influence the lipophilic/hydrophilic balance of the antioxidant and consequently its distribution between the aqueous and lipid phases. The amphiphilic apo-lycopenoic acids could access the heme crevice for a direct reduction of the hypervalent iron species involved in the initiation of the peroxidation process whereas the action of more lipophilic derivatives (aldehydes, ethyl esters) is likely restricted to the lipid phase (reduction of lipid peroxy radicals). Despite their high reducing activity, alcohols are weak inhibitors, possibly because of their fast conversion into inactive oxidation products

Mechanisms for cancer prevention by carotenoids and their derivatives

Yoav Sharoni and Joseph Levy

Faculty of Health Sciences, Ben-Gurion University of the Negev and Soroka Medical Center, Beer-Sheva, Israel. Email: yoav@bgu.ac.il

There is considerable epidemiologic evidence suggesting an association between the consumption of fruits and vegetables and reduced incidence of cancer. In particular, carotenoids have been implicated as cancer-preventive agents. Support for the anticancer effects of carotenoids was found with diverse cancer cells in vitro which allows also analysis of the mechanisms involved in this processes. In this presentation I will address some of these mechanisms focusing on the induction of phase II enzymes and the inhibition of sex hormone activity.

In recent years, evidence has accumulated indicating that the cancer preventive action of dietary ingredients in general and carotenoids in particular is, at least in part, due to the induction of phase II detoxifying and antioxidant enzymes. Expression of phase II enzymes is regulated by the antioxidant response element (ARE) and the transcription factor Nrf2. Dietary inducers of Nrf2 are diversified in their chemical structure but are usually electrophiles which react with Keap1 to disrupt its inhibitory activity on Nrf2. However, hydrophobic carotenoids such as lycopene lack any electrophilic group and thus are unlikely to interact directly with Keap1. Indeed, we have demonstrated that intact carotenoids do not directly activate the ARE transcription system and that carotenoid oxidation products are the active mediators in this stimulation. Furthermore, the activity of the carotenoid derivatives depends on the relative position of the methyl group to the terminal aldehyde which determines the reactivity of the conjugated double bond in reactions such as Michael addition to SH groups in Keap1.

Breast cancer is the most common cancer among women and there is substantial evidence to suggest that estrogens play an important role in both the development and progression of breast cancer as well as of endometrial cancer, and that estrogens increase the risk of developing these malignancies. Inhibiting estrogenic activity is a major strategy for treatment and prevention of these hormone dependent cancers. Thus, we aimed to determine whether carotenoids inhibit estrogen signaling in mammary and endometrial cancer cells, which could explain their cancer preventive activity. We found that tomato carotenoids (lycopene, phytoene, phytofluene, and β -carotene) inhibited estrogen-induced cell proliferation. The inhibition of cell growth by lycopene was accompanied by slow down of cell-cycle progression from G1 to S phase. Moreover, carotenoids inhibited estrogen-induced activation of the transcriptional activity of the estrogen receptors. Our studies suggest that the transcription factor Nrf2, is partially involved in the carotenoid inhibition of estrogenic activity in cancer cells.

Although the effect of estrogens in breast and endometrial cancer is harmful, it is beneficial for bone formation. Thus, we investigated the effect of the carotenoids and other phyto-nutrients on estrogenic activity in osteoblasts which are bone forming cells. To our surprise, the same dietary compounds, which inhibit estrogenic activity in cancer cells, did not inhibit and even stimulated the expression of estrogen-induced genes in the bone cells. As discussed

above, Nrf2 was found to be involved in the inhibition of estrogenic activity in breast cancer cells. In contrast, in bone cells, over-expression of Nrf2 enhanced estrogen-induced transcription, similar to the effect of the phyto-nutrient. In addition, reduction of Nrf2 level, by siRNA, leads to a decrease in phytonutrient supported activity of estrogens in bone cells. The results suggest that the Nrf2 transcription factor is involved in the opposite effects of carotenoids and other phytonutrients on estrogenic activity in breast cancer versus bone cells.

Lycopene is a potent activator of RAR-mediated response pathways in an RARE animal reporter mouse model

Gamze Aydemir¹, Eric Reynaud², Catherine Caris-Veyrat² and Ralph Rühl¹

1-Apoptosis and Genomics Research Group of the Hungarian Academy of Science and Laboratory of Nutritional Bioactivation and Bioanalysis, Department of Biochemistry and Molecular Biology, University of Debrecen, Hungary; 2-INRA, University of Avignon, Avignon, France

Lycopene, a non-provitamin A carotenoid, is commonly found in tomato and tomato based products. Increased consumption of lycopene has been associated with a decreased risk of cardiovascular diseases, lung cancer and prostate cancer. Little information is known about bioactivation of lycopene. The objective of our study is to investigate whether lycopene or its metabolites are potent transcriptional activators of nuclear hormone receptor mediated signalling. RARE-Luc mouse, engineered to express RARE (retinoic acid response element) under the control of firefly luciferase gene was utilised. Three different male mice groups were treated with ATRA (*all-trans* retinoic acid), lycopene and lycopene beadlets to examine organ specific luciferase expression. 100 mg / kg lycopene was given by oral gavage, and compared to mice groups supplemented with ATRA (50 mg / kg) and lycopene vehicles (100 mg / kg). A high basal level of bioluminescence signal was observed in testis and brain. After lycopene treatment, bioluminescence signal significantly increased in white adipose tissue (WAT), spleen, large intestine, lung, liver and kidney. Furthermore, RARE-Luc mice were supplemented with apo-10' and 14'-lycopenoic acid by oral gavage and compared with DMSO treated group. Results displayed that apo-10'-lycopenoic acid can activate the RARE in prostate and lung significantly whereas apo-14'-lycopenoic acid can induce the transcriptional activation via RAR mediated pathways in brain, intestine, large intestine, spleen, prostate, thymus, WAT, liver and kidney.

RARE-Luc mouse is kindly provided from Prof. Dr. Rune Blomhoff, Oslo, N.

LYCOPENE AND CHEMOTHERAPY TOXICITY

Kazim Sahin¹, Nurhan Sahin¹, Omer Kucuk²

¹Firat University, Elazig, Turkey; ²Emory University, Winship Cancer Institute, Atlanta, Georgia, USA

Antineoplastic agents induce oxidative stress i.e., the production of free radicals and other reactive oxygen species (ROS) in biological systems. Oxidative stress reduces the rate of cell proliferation, and that occurring during chemotherapy may interfere with the cytotoxic effects of antineoplastic drugs, which depend on rapid proliferation of cancer cells for optimal activity. Reactive oxygen species contribute to side effects that occur only with individual agents, such as doxorubicin-induced cardiotoxicity, cisplatin-induced nephrotoxicity, and bleomycin-induced pulmonary fibrosis. Tomato and tomato-based products are an important dietary source of antioxidants such as lycopene, b-carotene, α -tocopherol and phytoene, and phytofluene. Lycopene is a major carotenoid present in tomatoes and highly potent antioxidant that provides protection against cellular damage caused by reactive oxygen species. It is one of the most potent antioxidants among dietary carotenoids. Lycopene can reduce or prevent many of side effects of chemotherapy, and the protective effect results from activities other than their antioxidant properties. This review focuses on recent investigation into the protective effect of lycopene against antineoplastic agents-induced lipid peroxidation and toxicity in vivo and in vitro studies.

Preventive role of lycopene on oxysterol-induced pro-inflammatory cytokine cascade in human macrophages

Rossella Simone¹, Assunta Catalano¹, Nadia Parrone¹, Giovanni Monego², Franco O. Ranelletti³, and Paola Palozza¹

¹Institute of General Pathology, ²Institute of Anatomy, ³Institute of Histology, Catholic University-School of Medicine, Rome, Italy

It is now well accepted that oxysterols play important roles in the formation of atherosclerotic plaque, involving cytotoxic, pro-oxidant and pro-inflammatory processes. It has been recently suggested that tomato lycopene may act as a preventive agent in atherosclerosis, although the exact mechanism of such a protection is not clarified. The main aim of this study was to investigate whether lycopene is able to counteract oxysterol-induced pro-inflammatory cytokines cascade in human macrophages, limiting the formation of atherosclerotic plaque. Therefore, THP-1 macrophages were exposed to two different oxysterols, such as 7-keto-cholesterol (7-KC) (10-20 μM) and 25-hydroxycholesterol (25-OHC) (2-4 μM), alone and in combination with lycopene (0.5-2 μM). Both oxysterols enhanced pro-inflammatory cytokine (IL-1 β , IL-6, IL-8, TNF α) secretion and mRNA levels in a dose-dependent manner, although at different extent. These effects were associated with an increased reactive oxygen species (ROS) production through an enhanced expression of NAD(P)H oxidase (NOX-4). Moreover, a net increment of phosphorylation of ERK1/2, p-38 and JNK and of nuclear factor kB (NF-kB) nuclear binding was observed. Lycopene prevented oxysterol-induced increase in pro-inflammatory cytokine secretion and expression by inhibiting oxysterol-induced ROS production and by reducing the phosphorylation of MAPKs and NF-kB activation. Moreover, the carotenoid increased PPAR γ levels in THP-1 macrophages. Taken all together, these data bring new information on the anti-atherogenic properties of lycopene, and on its mechanisms of action in atherosclerosis prevention.

Processed tomatoes on vasodilatation and C-reactive protein (hsCRP) in overweight and obese men and women

Rose Giordano¹, Tissa Kappagoda², Mandeep Cheema¹, Gwen Young⁵, Jack Cappozzo⁴, Yumei Cao³, Penny Kris-Etherton³, Britt Burton-Freeman⁴ *Depts. of Nutrition¹ and Internal Medicine², University of California, Davis, CA 95616, Penn State University, University Park, PA 16802³ and The National Center for Food Safety and Technology 6502 S. Archer Road Summit-Argo, IL 605014⁴, Kagmoe USA Inc⁵*

BACKGROUND: Overweight people are at risk for developing metabolic syndrome, which is associated with elevated inflammatory markers and reduced vascular endothelium function (EF). Presently, there is considerable interest in the role of the dietary carotenoids to improve the metabolic profile. Processed tomato products are known for their carotenoid content, particularly lycopene and they are relatively convenient, versatile, and accessible to most people. In this trial, the relationship between forearm mediated dilation (FMD) during acute and chronic consumption of high tomato (TOM) vs placebo meal (Pbo) and inflammatory markers will be evaluated. We hypothesized that processed tomatoes will reduce markers of chronic inflammation and the acute inflammatory response to a high fat meal (HFM) in overweight subjects.

METHODS: Processed tomato- or non-tomato products matched in energy, sodium, and sugar content (high tomato HT, 5 servings/d vs low tomato LT, <1 servings/d) were included in the diets of 56 free-living individuals (Age=44±12 yr, F=35, M=21) and tested in a multi-center, randomized, controlled trial. All subjects had a 3 wk LT run-in followed by either 6 wk HT or LT diet. A random subset of 23 subjects had 3 postprandial (pp, 6hr) visits; at wks -1 and 0 (run-in) and at 6 wk to assess the acute and residual affects of tomato vs non-tomato diet on meal-induced post-prandial inflammation, as measured by hsCRP and vaso-relaxation, as measured by flow mediated vasodilatation (FMD).

RESULTS: hsCRP was lower in overweight women (OW, BMI 25-29.9; HFM:1.66±0.15, Pbo:2.37± 0.21 mg/L, p=0.006) and obese men (Ob, BMI >30; HFM:3.1±0.21, Pbo:4.39±0.28 mg/L, p=0.006) after the HFM with tomato compared to no tomato during run-in. In addition, after 6 wk HT vs LT, hsCRP was lower in response to HFM (HT:1.98 ±0.15, LT:2.93± 0.27 mg/L, p=0.013). FMD was not different, possibly related to BMI (BMI by diet interaction, p=0.01).

CONCLUSION: These findings suggest that processed tomatoes have a role in modifying inflammatory status; increased adiposity (i.e. obese) may negate the benefits of diet on EF. Funding and tomato products provided by the Tomato Products Wellness Council.

LYCOPENE AND ENDOTHELIAL FUNCTION

PhD-Student Janine Kalkowski

Cardiologic research laboratory

Medical Clinic for Cardiology and Angiology, Campus Mitte,

Charité – University Medicine Berlin, Germany

Charitéplatz 1, D-10117 Berlin, Germany, Fax.: +49 -30 450-513941, Phone: +49 -30 450-513174,

E-mail address: janine.kalkowski@charite.de

The endothelium plays a crucial role in cardiovascular homeostasis mainly the vascular tone by releasing vasodilator substances, such as nitric oxid (NO). In normal endothelial function NO is produced in endothelial cells by the endothelial nitric oxide synthase (eNOS) and diffuses to the smooth muscle cells (SMC). NO activates smooth muscle soluble guanylyl cyclase to form cGMP which acts as a second messenger to mediate relaxation. In endothelial dysfunction less NO is bioavailable due to a reduced synthesis by eNOS or increased oxidative inactivation of NO by reactive oxygen species (ROS). ROS are generated in large amounts in atherogenic processes within the artery wall by macrophages and foam cells that accumulate at lesion sites. This initial step of atherosclerosis progression is characterized by detrimental functional consequences like endothelial dysfunction and vascular remodeling. Antioxidants have the ability of decreasing ROS-induced oxidative damage and preventing LDL oxidation in early stages of atherogenesis. Lycopene has attracted much attention as a dietary antioxidant.

Several animal studies have been performed to address the anti-atherogenic effects of lycopene. Atherogenesis has been induced in laboratory animals by administration of a cholesterol-enriched diet alone or in combination with oil or egg yolk. These studies demonstrated increased blood lipid levels, in particular LDL-cholesterol level, as well as plaques and endothelial dysfunction in later stages. One study investigated the effect of lycopene on atherosclerosis induced by a high-cholesterol diet in NZW rabbits. They found decreased NO levels after high-cholesterol diet but not after such a diet with additional lycopene administration. This finding demonstrates a hint of impaired endothelial function in the cholesterol group which is potentially restored after co-treatment with lycopene. Our results of endothelial function measurements in isolated aortic and carotid artery rings of male NZW rabbits that were fed a standard diet, a high-cholesterol diet or a high-cholesterol diet plus lycopene showed a moderately improved endothelial function after lycopene treatment.

Epidemiological human studies have shown that lycopene could play a role in the prevention of cardiovascular diseases. Dietary intervention studies have shown short-term reduction of LDL oxidation after lycopene supplementation. Furthermore, low concentrations of serum lycopene have been associated with increased intima-media thickness of the carotid artery wall. Kim et al. found this effect as an independent inverse relationship between circulating lycopene and arterial stiffness. Those findings suggest a preventive role of lycopene in cardiovascular disease and atherosclerosis.

In a randomized cross-over dietary intervention study performed by our group, we supplemented 19 postmenopausal women with lycopene for one week and measured endothelial function. We observed an increase in lycopene plasma levels compared to baseline and the control group. However endothelial-dependent vasodilatation remained unaffected.



Posters
Abstracts

Tomato, Lycopene and Health Workshop

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INCORPORATION OF VEGETABLE JUICE INTO A DIET CAN REDUCE THE GAP BETWEEN VEGETABLE INTAKE AND DIETARY RECOMMENDATIONS AND IS ASSOCIATED WITH IMPROVED RISK FACTORS LINKED TO VASCULAR DISEASE

Kazaks AG¹, Holt RR¹, Shenoy SF¹, Foreyt JP², Reeves RS², Winters BL³, Khoo CS³, Poston WSC⁴, Haddock CK⁴, Keen CL*¹

¹University of California, Davis, CA, USA; ²Baylor Medical College, TX, USA; ³Global Nutrition and Health, Campbell Soup Company, NJ, USA; ⁴HOPE Health Research Institute, MO, USA.

Programs aimed at improving daily vegetable intake have had limited success. Randomized controlled trials were used to test whether providing a vegetable juice (VegJ), in addition to counseling on the Dietary Approaches to Stop Hypertension (DASH) diet, would increase vegetable intake and improve health parameters in two separate study populations: 1) General Population (GP, age 50.5 years) and 2) Metabolic syndrome population (MetS, age 49.8 years). Both study populations received instruction on the DASH diet, with an emphasis on increasing vegetable consumption. The MetS group DASH instructions were also calorie controlled for weight loss. Both studies had a control group (no VegJ), and were 12 weeks in duration. **Results:** The GP group was well educated (62% had a master's, doctoral or professional degree), while education level was lower in the MetS group (59% completed high school or less). Despite DASH diet counseling, with specific encouragement to increase vegetable intake, neither group met public health recommendations of at least 2 cups (4 servings) per day/2000 Kcal diet. However, when participants in both groups were provided a tangible product, either regular or low sodium VegJ, they increased their vegetable intake to recommended levels (GP 2.8 ± 0.1 cups; MetS 3.2 ± 0.2 cups, mean \pm SEM). The subjects with MetS who consumed 1-2 cups of the VegJ/day lost significantly more weight than their no VegJ controls (-1.8 ± 0.4 vs. -0.6 ± 0.6 kg, mean \pm SEM respectively, $p=0.03$, Intention-to-Treat model) who received the same DASH dietary counseling with an emphasis on vegetables. In the GP study, a subset of subjects with pre-hypertension had significant reductions in systolic, diastolic and mean arterial blood pressures after 12 weeks of VegJ consumption ($p<0.05$). **Conclusion:** Results from these two intervention studies support the concept that, in different population groups, tangible health benefits can arise with a modest increase in vegetable intake. The results suggest that individuals will make behavior changes that can improve vegetable intake when simple, practical approaches are available.

Supercritical CO₂ extraction of lycopene from tomatoes: a procedure in obtaining a unique natural product.

Marcello Salvatore Lenucci^{*}, Leonardo Rescio^{**}, Gabriella Piro^{*} and Giuseppe Dalessandro^{*}.

^{*} Di.S.Te.B.A., Università del Salento, Via prov. Lecce-Monteroni, 73100 Lecce, Italy

^{**} Pierre S.r.l., S.P. 362 Km 17,650, 73013 Galatina (LE), Italy

Lycopene is a carotenoid found in a wide variety of vegetables and fruits. There are more than six hundred natural carotenoids, but only twenty of those have been found in human blood and tissues. Lycopene is the most abundant carotenoid in human blood, followed by β -carotene, lutein and zeaxanthin.¹ The purpose of this work was to obtain a natural, solvent-free lycopene extract from red-ripe tomato fruits. This project has been developed in three different steps finalised to control and optimise the entire productive cycle from fresh tomatoes to the production of organic lycopene. The first step was the accurate selection, experimentation and production of organic grade certified tomato varieties characterised by high-lycopene content,² followed by the biochemical analyses of their antioxidant characteristics.³ The second step was the set-up of a process to obtain a freeze-dried tomato matrix suitable for the extraction by supercritical carbon dioxide (SC-CO₂) from the new selected high-lycopene varieties.⁴ The last step was the optimization of SC-CO₂ lycopene extraction from a defined blend of the tomato matrix with a co-matrix composed of roughly crushed hazelnut seeds. This procedure allowed the simultaneous extraction of lycopene from the tomato matrix and oil from hazelnut co-matrix which improves lycopene solubility and increase the yield of recovered end-product, according to the Pierre S.r.l. Galatina patented co-extraction technology.^{5,6} The extract is an over-saturated solution of lycopene in hazelnut oil (oleoresin). This innovative procedure has permitted to realise a high lycopene extraction yield. The oleoresin represents a healthy and high added-value source of lycopene, suitable for nutraceutical, cosmetic and pharmaceutical purposes. It was patented as the first organic grade lycopene. This innovative product is characterized by: 1) the absence of toxic impurities such as solvents, pesticides, heavy metals and dioxins; 2) a high natural bioavailability, improved by the presence of the co-extracted natural compounds from tomatoes and hazelnuts; 3) a high antioxidant activity, due to the presence of other carotenoids from tomato and tocopherols and phyosterols from the hazelnut seeds.

¹Rao, 2006, Caledonian Science Press: Badalona, Spain; ²Cantore et al., 2008, *Acta Hort* 789:173-180; ³Lenucci et al., 2006, *J Agr Food Chem* 54:2606-2613; ⁴Lenucci et al., 2007, *Acta Hort* 758:173-180; ⁵Vasapollo et al., 2004, *J Supercrit Fluid* 29:87-96; ⁶Ciurlia et al., 2009 *J. Supercrit Fluid* 49:338-344.

Lycopene Formation in *Blakeslea trispora*. Yield, Selectivity and Safety Aspects

Fani Mantzouridou and Maria Z. Tsimidou
Laboratory of Food Chemistry and Technology, School of Chemistry,
Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.
Email: tsimidou@chem.auth.gr; fmantz@chem.auth.gr

ABSTRACT

The biological role of lycopene in human health has stimulated interest in its commercialization by the food industry (e.g. 1). Lycopene by microbial sources is an alternative natural product considering that its exclusive source till very recently was tomato (2). The present work is focused on the current knowledge about lycopene production by *Blakeslea trispora* cells with regards to essential structural characteristics of the bioregulators used the last forty years (3). Moreover, needs that should be addressed in future trials so that the safe character of the end product to be ensured are also highlighted.

Taking into account the relative effectiveness and toxicity of various lycopene cyclase inhibitors applied so far we introduced 2-methyl imidazole to reduce risks of this source of hazard for the bioprocess (4). Optimization of the bioprocess conditions was based on response surface methodology to achieve maximum lycopene production. In the course of testing carotenogenesis regulation and lycopene yield, 2-methyl imidazole was fed into the fermentation broth at a descending order of addition level (200, 100, 50, 10 mg/L of culture medium). The optimized bioprocess in the presence of 2-methyl imidazole at 50 mg/L of culture medium yielded to 2.5% of lycopene (94% of total carotenoids) in fungal biomass. The lycopene yield is 360 mg/L of culture medium. Taking into account that tomatoes contain 0.05–0.1% of lycopene in their dry biomass, the prospective of 2-methyl imidazole on lycopene production in future trials in both laboratory and industrial scale should be considered a promising area of research. Comparative studies on chemical properties of microbial lycopene and lycopene from tomato is of special scientific interest.

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Tomato extract supplemented diet: effect on egg yolk pigmentation and lycopene crossing-over.

Strazzullo G.¹, Rotolo L.², Pagella M.², Brugiapaglia A.³, Schiavone A.²

¹Consiglio Nazionale delle Ricerche, Istituto di Scienze delle Produzioni Alimentari;

²Università degli Studi di Torino, Dipartimento di Produzioni Animali Epidemiologia ed Ecologia;

³Università degli Studi di Torino, Dipartimento di Scienze Zootecniche,
Via Leonardo da Vinci, 44 – 10095 Grugliasco (TO), Italy

INTRODUCTION - Lycopene is the principal carotenoid in red tomatoes and it's a natural pigment commercially used. Several studies have shown that lycopene dietary intake provide positive effects in human health for it's antioxidant activity. The aim of this study is to verify the ability of hen to transfer lycopene from feed to egg using tomato pulp extract as lycopene source.

MATERIALS AND METHODS - 20 Isa Brown laying hens were individually cages and were randomly divided in two groups of ten layers. C group was fed with a standard layer diet and was compared with TE group fed with the same diet supplemented with tomato extract (TE group) containing 13.2 mg lycopene/kg diet. Feed and water was provided ad libitum. San Marzano (high content lycopene cultivar) canned tomatoes were processed and lipophilic extract (red organic layer) was obtained as described by Strazzullo *et al.* 2007. The experimental period lasted 14 days and all the eggs were collected on days 1,7 and 14 of feeding. Yolks were weighed and colour was measured by Cielab system. Lycopene amount in egg yolk was determined by HPLC based method. Data were analyzed by ANOVA using GLM procedure of SPSS considering the dietary treatment as fixed factor.

Table 1. CIELAB parameters and lycopene content in egg yolks

	Day 1			Day 7			Day 14		
	C	TE	P	C	TE	P	C	TE	P
L*	60.9 ± 2	60.9 ± 0	N	62.1 ± 3	58.0 ± 5	≤ 0.05	63.4 ± 8	60.7 ± 1	≤ 0.01
a*	14.0 ± 2	13.9 ± 8	N	14.4 ± 6	16.8 ± 5	≤ 0.01	14.4 ± 5	16.5 ± 1	≤ 0.01
b*	34.8 ± 4	34.8 ± 5	N	35.3 ± 8	34.0 ± 0	≤ 0.05	35.9 ± 0	34.5 ± 5	≤ 0.05
Lycopene	N.D.			N.D.	0,12 ± 0,03		N.D.	0,16 ± 0,06	

RESULTS AND DISCUSSION - The pigmenting effect of lycopene determined significantly higher a*(redness) value (P≤0.01) on days 7 and 14 for TE group and lowers L* and b* values on the same days. HPLC analysis confirmed lycopene crossing-over from fed to egg yolk on TE group on days 7 and 14, with 0,12 µg/g and 0,16 µg/g respectively (Table 1). Mean transfer efficiency resulted to be 1,06 %. Data recorded suggested that the use of tomato may have positive influence on yolk pigmentation, increasing the redness value. Lycopene crossing-over observed is comparable with others researches (Knoblich *et al.* 2005).

CONCLUSIONS – Yolk colour is an important factor in determining acceptability of egg. This research confirmed that dietary intake of tomato resulted in lycopene transfer that affect yolk colour. This is an important consideration because recently, consumers have become more concerned about the use of alternative feeds, and tomato extract supplemented egg could be comprised in functional foods with health-promoting properties as carotenoid enriched product.

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Lycopene supplementation regulates oxidative stress markers and expression of caspases, Bax, Bcl-2 and in 7,12- dimethylbenz[a]anthracene-induced liver damage in rats

M. Tuzcu^a, Can A. Agca^a, F. Akdemir^b, N. Sahin^b, K. Sahin^b, M. Onderci^c, S. Seren^d, O. Kucuk^e

^a Department of Biology, Faculty of Science Firat University, 23119 Elazig, Turkey

^b Department of Animal Nutrition, Faculty of Veterinary Science, Firat University, 23119 Elazig, Turkey

^c Department of Biochemistry, Health Collage, Adiyaman University, Turkey

^d Wayne State University, Detroit, MI, USA

^e Emory University, Winship Cancer Institute, Atlanta, Georgia, USA.

Lycopene is a carotenoid compound found in certain fruit, such as tomato, watermelon, pink grapefruit and guava in high concentrations. Dietary intake of lycopene is inversely associated with the risk of cancer. The present study was conducted to the effects of lycopene on the oxidative stress markers and expression of bax, bcl-2 and caspases during 7,12-dimethylbenz[a]-anthracene (DMBA)-induced liver damage in Wistar albino rats. Thirty (8 weeks-old) Wistar albino rats were assigned to 3 experimental groups containing 10 rats in each. Group I served as normal control animal. Group II animals received 7,12-dimethylbenz[a]anthracene (DMBA) (50 mg/kg body weight of DMBA in olive oil on day 1 and day 30 of the experiment) i.p. to develop liver damage. Group III received DMBA i.p. to develop liver damage in animals treated with lycopene (20 mg/kg body weight daily, suspended in olive oil, by gavages) starting from day 1 for a total of 20 weeks; while the rats in DMBA and control groups received the same dose of olive oil without lycopene daily for 20 weeks. In the DMBA group, biomarkers of oxidative stress of liver (MDA, 8-isoprostane) were significantly increased, GSH levels were decreased. However, the rise in oxidative damage was significantly reduced and GSH was increased by lycopene supplementation. In addition, in the DMBA-treated rats, apoptosis was induced by a decrease in expression of bax and an increase in expression of Bcl-2. These changes were restored to their normal values in the lycopene-treated rats. Upregulation of caspase-3 and caspase-9 was also increased by lycopene. These results suggest that lycopene exerts a hepatoprotective effect on rats with DMBA-induced oxidative stress through its antioxidant activity and the reduction of apoptosis.

Extraction of lycopene from tomato using supercritical CO₂ and vegetable oil as co-solvent.

Giuseppe Vasapollo¹, Luigia Longo¹, Leonardo Rescio², Loredana Ciurlia²

¹Dipartimento di Ingegneria dell'Innovazione, Università di Lecce, Via Arnesano 73100 Lecce, Italy; ²PIERRE CHIMICA S.r.l., s.s. 476 Km 17,650 Zona industriale, 730213 Galatina (LE), Italy

Lycopene (Fig. 1) is natural compound insoluble in water, soluble in organic solvents, decomposes easily during the time, used in the dyeing of different kinds of foodstuffs [1]. This work describes the extraction of lycopene using supercritical CO₂ from tomato and hazel-nut oil as co-solvent. Supercritical CO₂ is an economic, non-toxic and non-flammable solvent, easily recyclable and separable after processing due to its volatility. Thus, the extraction of lycopene was carried out with co-solvent at pressure and temperatures ranging from 335 to 450 bar and 45 to 70 °C respectively and the experiments have shown that the amount of extractable lycopene depended on the experimental conditions and have demonstrated that the presence of the co-solvent improved the yields of the lycopene extract and had a beneficial role in terms of stability of the pigment. The maximum amount of the extractable lycopene from dried and grinded tomato at 450 bar and 66 °C in the presence of co-solvent and utilizing a flow rate of about 20 KgCO₂/h was almost 60%. The extract were analysed by high-performance liquid chromatography and UV-Vis spectra.

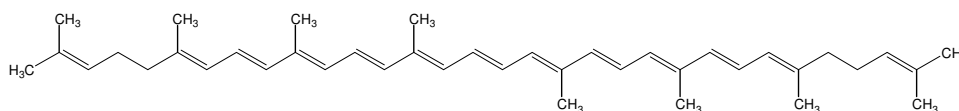


Fig. 1. Lycopene

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