Results from LYCOCARD project
Food product studies
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The LYCOCARD project in brief

LYCOCARD investigates the role of lycopene in reducing the risk of cardiovascular diseases

GOALS:
• Establish improved nutritional guidelines (help consumers to select healthy foods)
• Design and development of healthy new foods based on tomatoes (strengthen food industry)

Specifically points to clarify the following:
1. Effects of technological processing on lycopene
2. Interactions between different food ingredients
3. Molecular aspects of absorption and metabolism of lycopene
4. Biological effects of lycopene isomers and lycopene metabolites.
The LYCOCARD project in brief

**In vitro studies**
- CHARITE BERLIN
- UNI DEBRECEN
- UNI LIVERPOOL
- INRA MARSEILLE
- INRA AVIGNON
- UNI ROME
- JUVER
- AGRAZ
- CONESA
- CONSERVE ITALIA

**In vivo studies**
- UNI JENA (co-ordination)
- NUTRIUNIT ROME
- NUTRIUNIT ROME
- HERZSTIFTUNG
- AMITOM

**Food product studies**
- CONSERVE ITALIA

**Nutritional guidelines & Dissemination**
- CALEDONIAN SCIENCE PRESS
Objectives:

• To analyze the bioactive compounds in tomatoes

• To analyze the effect of processing and storage

• To develop new tomato-based products for nutritional improvement
TOMATO BIOACTIVE COMPOUNDS

ANTIOXIDANTS

- Carotenoids
- Phenolic compounds
- Vitamin C
- Vitamin E

Antioxidant activity ($\Theta$ oxidative stress)
- $\downarrow$ CVD
- $\downarrow$ Cancer
- $\downarrow$ Inflammatory diseases
- $\downarrow$ Neurodegenerative diseases

NON ANTIOXIDANTS

- Folates

Essential vitamins (B9-Folic acid)

**Deficiency $\rightarrow$ $\uparrow$ Risk of:**
- Neural tube defects
- Colorectal cancer
- CVD ($\uparrow$[homocysteine] > 15 μM)

CVD risk factor: A 5 μM homocystein increase raises the risk of CVD by ~ 20% (Ueland 2000, Ford 2002)
EXPERIENCE 1

Characterization of tomato varieties for industrial use
EXPERIENCE 1

Characterization of tomato varieties for industrial use

Data on 36 tomato cultivars:

<table>
<thead>
<tr>
<th>Lycopene content</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6-8 mg/100g</td>
<td>10-11 mg/100g</td>
<td>12-14 mg/100g</td>
</tr>
<tr>
<td>REVOLUTION (6.8)</td>
<td>MALVA (10)</td>
<td>ASPEN (12)</td>
</tr>
<tr>
<td>JERIEX (7)</td>
<td>CAPEA (10)</td>
<td>H-9997 (13)</td>
</tr>
<tr>
<td>HIPACK (8)</td>
<td>H-9665 (11)</td>
<td>CDX-255 (14)</td>
</tr>
</tbody>
</table>

Comparison of total lycopene content in different cultivars from 2006 to 2008
EXPERIENCE 1

Characterization of tomato varieties for industrial use

Total lycopene

Seasonal variations

Phenolics

Folates
EXPERIENCE 1

Characterization of tomato varieties for industrial use

Changes in bioactive compounds during ripening

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**Lycopene**

![Lycopene Chart]

**Total phenolic compounds**

![Total Phenolic Compounds Chart]
EXPERIENCE 2

Effect of processing and storage on bioactive compounds
EXPERIENCE 2

Effect of homogenization and thermal processing

Raw tomato puree (RTP)

HOMOGENIZATION

<table>
<thead>
<tr>
<th>ONE-STEP</th>
<th>TWO-STEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>H100 bar</td>
<td>H150+50 bar</td>
</tr>
<tr>
<td>H150 bar</td>
<td>H100+100 bar</td>
</tr>
<tr>
<td>H288 bar</td>
<td>H190+20 bar</td>
</tr>
</tbody>
</table>

PASTEURIZATION (98°C/40 s)

Mean losses after thermal treatment:
- Lycopene (no significant changes)
- Total phenolics and vitamin C (10-15%)
EXPERIENCE 2

Stability of tomato bioactive compounds during storage

TOMATO JUICE

TETRAPACK

GLASS

LYCOPENE
TOTAL PHENOLICS
TOTAL FLAVONOIDS
VITAMIN C
FOLATES
TOTAL ANTIOXIDANT ACTIVITY (HYDRO- & LIPOPHILIC)

8°C  22°C  37°C  8°C  22°C  37°C

STORAGE FOR 12 MONTHS
EXPERIENCE 2

Stability of tomato bioactive compounds during storage

LYCOPENE AND ITS ISOMERS

LYCOPENE ISOMERS (Tetrapack)

LYCOPENE ISOMERS (Glass)
EXPERIENCE 2
Stability of tomato bioactive compounds during storage

LYCOPENE AND ITS ISOMERS

Total lycopene losses at the end of the storage trial <17%

Literature survey:
Reported lycopene losses 30-75% (tomato paste, tomato juice, canned tomato) 3 mo/4°C
EXPERIENCE 2
Stability of tomato bioactive compounds during storage

TOTAL PHENOLICS AND FLAVONOIDS

TOTAL PHENOLICS (Tetrapack)

0 2 4 6 8 10 12
0 50 100 150 200 250 300 350
Storage time (months)
mg GAE/kg

TOTAL FLAVONOIDS (Tetrapack)

0 2 4 6 8 10 12
0 25 50 75 100 125
Storage time (months)
mg CE/kg

TOTAL PHENOLICS (Glass)

0 2 4 6 8 10 12
0 50 100 150 200 250 300 350
Storage time (months)
mg GAE/kg

TOTAL FLAVONOIDS (Glass)

0 2 4 6 8 10 12
0 25 50 75 100 125
Storage time (months)
mg CE/kg
EXPERIENCE 2

Stability of tomato bioactive compounds during storage

- No vitamin C detected in glass-bottled juice
- Vitamin C is ADDED to the juice in Tetrapack (Vitamin C-enriched)
- Stability depends on storage time & temperature

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>% losses</th>
</tr>
</thead>
<tbody>
<tr>
<td>8°C</td>
<td>45%</td>
</tr>
<tr>
<td>22°C</td>
<td>65%</td>
</tr>
<tr>
<td>37°C</td>
<td>85%</td>
</tr>
</tbody>
</table>
EXPERIENCE 3

Development of new tomato-based products
EXPERIENCE 3

Development of new tomato-based products

**Ketchup with improved nutritional profile**
- More tomato (lycopene)
- Less sugar
- Less salt (Na⁺)

**Tomato juice enriched with vitamin C and folic acid**
- 20 mg lycopene/serving (200 mL)
- Improved lycopene absorption (2 homogenization steps)
- Cover 60% of RDA* vitamin C and folic acid/serving

*Commission Directive 2008/100/EC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions.

**In vivo**
(human studies)

Friedrich-Schiller-Universität Jena

Human feeding studies still in progress
SUMMING UP

- Bioactive compounds markedly affected by cultivar and year of harvest.

- Highest levels of lycopene in full-ripe tomatoes but not all the biologically active compounds necessarily increase in tomatoes harvested at full ripeness.

- 2-step homogenization led to increased lycopene isomerization and folate release from tomato matrix → Design of new products.

- Lycopene and phenolic compounds remain essentially stable during storage.

- Vitamin C and folates are the most labile compounds and they are markedly affected by storage conditions.

- Room temperature (22°C) is appropriate to keep lycopene and phenolic compounds. As for Vitamin C and folates even refrigeration temperatures lead to important losses.

- Tetrapack material better preserves folates but the use of glass or tetrapack makes no difference in the case of lycopene and phenolic compounds.
Related publications:

• Pérez-Conesa et al. (2009). *IFSET.* 10:179-188
Ralph Rühl
Presentation Estoril 2010

“Tomatoes, lycopene and CVDs"
Aim of LYOCARD

LYOCARD®
BRIDGING THE GAP

HEALTHY FOOD
BIOACTIVE CHEMICALS

FOOD PRODUCTS
HEALTHY DIETS
NUTRITIONAL GUIDELINES

HEALTH BENEFITS
DISEASE PREVENTION
Clinical studies within LYCOCARD

1. Rome study
   - healthy obese patients 100 g tomato passata per day for 9-15 months
   - no main alteration of inflammatory parameters
   - just acute phase response proteins are significantly down-regulated

2. Berlin study
   - human intervention study examining endothelial functions in healthy post-menopausal women with 70 g tomato puree (46 mg LYC) per day for 2 weeks
   - no significant alteration of endothelial function

3. Jena study (in process)
   - human intervention study with newly developed tomato products
   - tomato juice / tomato puree per day for 6 weeks (20 mg LYC per day)
   - in progress

4. Marseille study (in process)
   - post-prandial experiment with tomato puree-rich meal (50 mg LYC per day)
   - transcriptomics in adipose tissue, PBMCs
   - in progress

→ SO FAR NO REAL POSITIVE CORRELATION BETWEEN TOMATOES CONSUMPTION AND ATHEROSCLEROSIS
Additional value from LYCOCARD studies

1. Lycopene and adipose tissue (Marseille)
   - mouse study and cell culture study with lycopene
   - downregulation of proinflammatory markers IL1β, IL6, MCP1
   - does not modulate adipogenesis (adiponectin)

2. Lycopene in a rabbit atherosclerosis model (Berlin)
   - fat induced atherosclerosis in NZW rabbits
   - no alteration in plaque volume
   - cholesterol and LDL-cholesterol significantly lower

3. Lycopene and nuclear receptors (Debrecen)
   - reporter animal and reporter cell culture studies
   - lycopene has strong pro-vitamin A activity and also activates nuclear receptors responsible for lipid homeostasis and metabolism

4. Lycopene and cell cycle / apoptosis (Rome)
   - lycopene can interfere with cell cycle and induce apoptosis
   - importance for cancer
1. **Lycopene on low-grade inflammation**
   - potential long term protective effects in diseases in which involve chronic inflammation like diabetes, atherosclerosis, atopic diseases (asthma), chronic lung diseases (COPD, asthma, etc.)
   -> **lycopene has the potential to ameliorate various diseases in which chronic inflammation plays an important role**

2. **Lycopene on diabetes**
   - lycopene is active in obese people to down-regulate pro-inflammatory markers. This is one major trigger leading from obesity to diabetes.
   -> **lycopene has the potential to ameliorate or even stop this process**

3. **Lycopene and cancer**
   - lycopene has been shown to interfere with cell cycle / apoptosis
   -> **lycopene has a potential to ameliorate or even reverse various kinds of cancers (prostate)**

4. **Lycopene and asthma**
   - lycopene and inflammation in the lungs
   -> **lycopene might have the potential to ameliorate or even stop chronic inflammatory diseases in the lung (COPD, asthma)**
We still have serum and PBMCs from various LYCOCARD clinical studies!

1. **Lycopene on low-grade inflammation**
   - PBMCs from 2 clinical studies
   - QRT-PCR of inflammation markers
   - LC-MS determination of pro- / anti-inflammatory lipid mediators
   - LC-MS of nutritionally relevant lycopene metabolites

2. **Lycopene on diabetes**
   - QRT-PCR of diabetes markers
   - LC-MS determination of pro- / anti-inflammatory lipid mediators
   - LC-MS of nutritionally relevant lycopene metabolites

3. **Lycopene and asthma**
   - QRT-PCR of pro-inflammatory markers
   - LC-MS determination of pro- / anti-inflammatory lipid mediators
   - LC-MS of nutritionally relevant lycopene metabolites
   (animal study in process in our labs in Hungary)

-> **LYCOPENE / TOMATOES are / maybe protective against these diseases!**
KEY TARGETS FOR LYCOPENE / TOMATOES AND HEALTH:

Evaluate the human intervention studies for potential correlations:

1. Lycopene on low-grade inflammation and on lipid metabolism.
2. Lycopene on diabetes.
3. Lycopene on asthma.
Future perspectives

We need to understand how lycopene/tomatoes might act:

A. We need to identify nutritional-relevant metabolites!

B. We need mechanisms how they are synthesized and distributed!

C. We need response mechanisms how these lycopene metabolites activate nuclear hormone response pathways!

D. We need to understand how these lycopene metabolites interfere with lipid metabolism and inflammatory pathways!
1. **Lipidomics:**
   - A. Lycopene metabolites
   - B. Pro- and anti-inflammatory lipid mediators

2. **Reporter animal models:**
   - for nuclear receptors involved in lipid metabolism and inflammation

3. **Transcriptomics:**
   - Robotic QRT-PCR and arrays for:
     - lipid metabolism target genes
     - lipid transport target genes
     - pro- and anti-inflammatory targets
     - markers for asthma, diabetes and etc.
Take home message

1. Lycopene is no wonder-drug against atherosclerosis
2. Lycopene can inhibit / ameliorate low-grade inflammation
3. Lycopene can mediate (positively) pro-inflammatory lipid metabolism
4. Lycopene can be potentially beneficial for diabetes induction
5. Lycopene can be potentially beneficial for chronic inflammation in the lung (asthma, COPD, etc.)
5. Lycopene / tomatoes and prostate cancer (mechanism still unknown: maybe apoptosis / cell cycle control)
Thank you for your attention!!

rruehl@dote.hu
The LYCOCARD Industrial Platform: An opportunity to promote tomato products

Sophie Colvine
General Secretary, AMITOM
LYCOCARD

Dissemination targets

- Scientists
  - Research results
- Industry
  - Competitive advantage
- Health Profession
  - Nutritional guidelines
- General public
  - Increased consumption
LYCOCARD communication

Scientists
Industry
Health profession
General public

Conferences
Peer reviewed articles
Annual reports
Training

www.lycocard.com
www.tomatoandhealth.com
www.tomatoandhealth.com
Languages versions

- English: www.tomatoandhealth.com
- German: www.tomateundgesundheit.de
- Italian: www.pomodoroesalute.it
- Spanish: www.tomateysalud.es

Other languages possible
Promotion opportunities

Limitations on what companies can say

- strict health claim legislations
- consumers weary of companies’ claim

Opportunities for generic promotion

- EU flag
- LYCOCARD provides public focus and scientific credibility
- Independence and brand neutrality
- Link with cereals industry – other industries? (olive oil, …)
Proposed communication on packaging

For more information on the place of tomato products in the Mediterranean diet:
www.tomatoandhealth.com

We support international research on tomato:
www.tomatoandhealth.com
Research

- Opportunity to discuss results with scientists
- Work together to make research results more applicable for industry
- Members may decide to fund additional work (health claim application?)
LYCOCARD
Industrial Platform

Aims:

- To create a forum to discuss research results with LYCOCARD scientists and potentially commission further research
- To promote the general consumption of tomato products while helping members to get competitive advantage
- To forge closer relations within the industry and to foster strategic alliances with natural partners in the food industry that offer synergistic promotional opportunities - such as cereals, olive oil and other groups like patient care and regional health care.
Until March 2011, costs are:

- 1,500€ for SMEs
- 4,000€ for other first stage processors
- 10,000€ for larger companies

Amount tax deductible as communication expenses

Fund managed by University of JENA and released by agreement of a steering committee
Join them today!