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# FUNCTIONAL FOODS: SCIENTIFIC FOUNDATIONS AND OPPORTUNITIES FOR THE AGRO-FOOD SECTOR

Zaragoza (Spain), 3-7 April 2006

CENTRO INTERNACIONAL DE ALTOS ESTUDIOS AGRONOMICOS MEDITERRANEOS
INSTITUTO AGRONOMICO MEDITERRANEO DE ZARAGOZA

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Curso Avanzado

ALIMENTOS FUNCIONALES: BASES CIENTÍFICAS Y OPORTUNIDADES PARA EL SECTOR AGROALIMENTARIO

Zaragoza (España), 3-7 abril 2006

**Francisco Javier Señoráns**
Dept. of Food Science
Universidad Autónoma de Madrid

# FUNCTIONAL FOODS: SCIENTIFIC FOUNDATIONS AND OPPORTUNITIES FOR THE AGRO-FOOD SECTOR

**Francisco Javier Señoráns**
Dept. of Food Science
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FUNCTIONAL FOODS: SCIENTIFIC FOUNDATIONS AND OPPORTUNITIES FOR THE AGRO-FOOD SECTOR

## Functional ingredients and their effects:

## Lipophilic Ingredients from vegetable products

**Dr. Francisco Javier Señoráns**
Dept. of Food Science
Universidad Autónoma de Madrid (Spain)

## Lipidic Ingredients from vegetable products

Tocopherols Sterols Carotenoids

Other antioxidants

What are they? Structure - types-function

Why functional ingredients? (bioactivity)

Do you have scientific evidence? Articles

Do you have clinical studies? Health claims

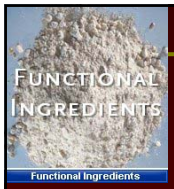
↓ OK, Lets do it

Where are they? Sources-industrial production

What functional food is the ideal for this ingredient? Available, compatible, complementary

Different cases:tocos-sterols-carots-etc





## Short introduction

## Functional Foods

**Son alimentos que tienen un efecto beneficioso sobre una o más funciones en el cuerpo, por encima y más allá de los efectos habituales de dicho alimento, tales como mejorar el estado de salud y bienestar o reducir el riesgo de enfermedad.**

**European International Life Sciences Institute (LSI)**

## Healthy foods, not functional foods

**NEW Functional Foods**
**Opportunities**

## Lipidic Ingredients from vegetable products

**Making of completely new Foods……**

**…or extraction of bioactive and health promoting ingredients from other products**



## Lipophilic Ingredients from vegetable products

Bioactive and health promoting ingredients from vegetable products

Potential functional food ingredients

## Bioactive and health promoting ingredients from vegetable products

1. FATTY ACIDS (OLEIC, LINOLENIC, OMEGA-3, ETC.)
2. TOCOPHEROLS
3. STEROLS
4. CAROTENOIDS
5. OTHER ANTIOXIDANTS: PHENOLIC DITERPENES
6. ISOFLAVONES AND ISOFLAVONOIDS
7. FLAVONOIDS
8. PHENOLIC COMPOUNDS
9. ORGANOSULFUR COMPOUNDS
10. ETC…



Which lipophilic plant ingredients ?

- TOCOPHEROLS
- STEROLS
- CAROTENOIDS
- OTHER ANTIOXIDANTS: PHENOLIC DITERPENES

## Lipophilic Ingredients from vegetable products

Which lipophilic plant ingredients ?

- **TOCOPHEROLS**
- **STEROLS**
- **CAROTENOIDS**
- **OTHER ANTIOXIDANTS: PHENOLIC DITERPENES**

Research and development on these ingredients ?



## Lipidic Ingredients from vegetable products

Tocopherols Sterols Carotenoids

Other antioxidants

What are they? Structure - types-function

Why functional ingredients? (bioactivity)

Do you have scientific evidence? Articles

Do you have clinical studies? Health claims

↓ **OK, Lets do it**

**Where are they? Sources-industrial production**

**What functional food is the ideal for this ingredient? Available, compatible, complementary**

**Produce and sell it !**

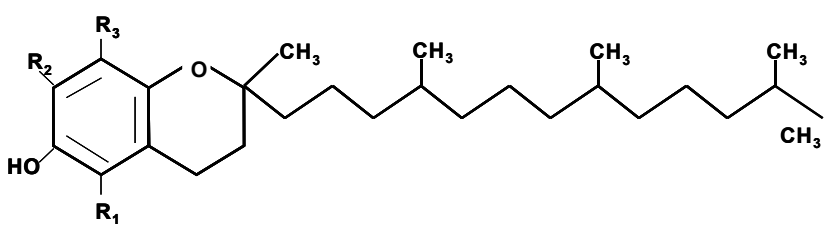
VITAMIN E…

…Functional food ingredient?



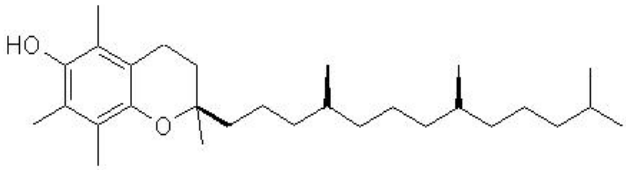
## Vitamin E

- The term vitamin E refers to a family of eight related compounds, the tocopherols and the tocotrienols
- The four major forms of vitamin E are designated α, β, δ, γ, on the basis of the number and position of the methyl group on the chromanol ring

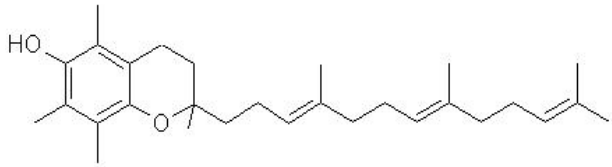


## Structure

•alpha-tocopherol




•alpha-tocotrienol

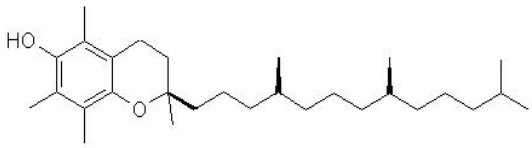





- Bioactivity of different Isomers: Vitamin E
  - 4 tocopherols + 4 tocotrienols

•alpha-tocopherol 3 chiral centers

•All Natural form are RRR




•Synthetic is all-rac-alpha-tocopherol (mixture of 8 isomers)

•Part of antioxidant network in biological systems (with Vitamin C)

## TOCOPHEROLS

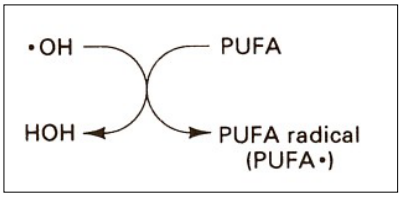
### Why functional ingredients? (bioactivity)

- **Biological effect: Vitamin E activity:**
  - **RRR-alpha-tocopherol 1.49 IU /mg highest activity**
- **Alpha-tocopherol ---highest bioavailability**
  - **predominant in human plasma and tissues**
  - **selective incorporation into nascent VLDL**

## TOCOPHEROLS

### Bioactivity and function

- **Antioxidant**
- **Free radical scavenger**
- Scavenge lipid peroxyl radicals
- Efficient oxygen quencher
  - Protects cell membranes
  - Protects LDL from oxidation
  - Prevention of rancidity
  - Works in conjunction with selenium



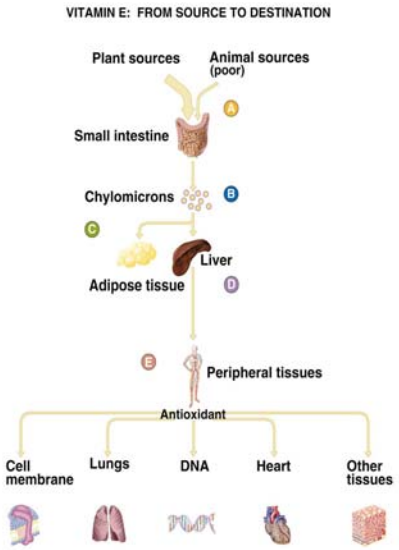

- Protection of double bonds in polyunsaturated fatty acids
  - Protects lungs from pollutants
  - Protects DNA
  - Protects heart
  - Others?

**Main activity of Tocopherols:**
**Most important lipid soluble antioxidant**

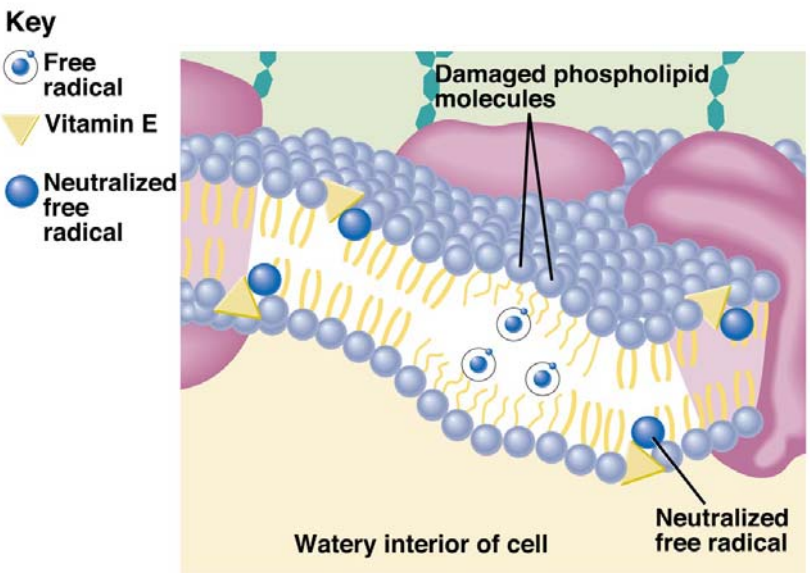
## TOCOPHEROLS

- **Health protection:**
  Stabilize reactions or situations that typically produce free radicals
  - Nucleic acid and protein metabolism
  - Mitochondrial metabolism
  - Supplementation may increase antibody production
  - When supplemented prior to slaughter, helps maintain meat quality (shelf life, color)

- Food protection:
  Main natural antioxidant in lipids




## TOCOPHEROLS



## *TOCOPHEROLS*

### Is there any scientific evidence?

- Chemical characterization and Different tests (activity *in-vitro*…): hundreds
- Epidemiological studies: several evidences
- Animal model tests (*in-vivo)*
- Pre-clinical:Dose effects, toxicological risks, etc.
- Clinical studies, Intervention studies: many

- Reliability: Total
- Controversial:
  - None on effect
  - Possible on need of supplementation
- Accepted: yes
- Approved health claims:
  - ??

## *TOCOPHEROLS*

### Are there clinical studies? Health claims

- Biological activity
  - Antioxidant

- Health promoting effects:
  - Protection against Atherosclerosis and Coronary Heart Disease
    - Reduce LDL oxidation : numerous studies (Lapointe, 2006)

  - Protection against some cancers: under study
    - hormonal, digestive tract…

- Recommended daily intake: 10 mg $\alpha$–tocopherol/day (15 IU)

- Supplementation 15-30 mg a-tocopherol/day to 100mg

- Related to intake of PUFA

## TOCOPHEROLS

### Do you have clinical studies? Health claims



Table 1
Studies investigating the effects of α-tocopherol supplementation on LDL oxidation parameters

| Authors (date) | Subjects' characteristics | Study design/duration | Dosage (per day) | Outcomes |
|---|---|---|---|---|
| Reaven et al. (1993) [66] | Mildly hyperlipidemic subjects ($n$=16) | Randomized<br>Free-living<br>Supplement provided<br>8 weeks | 1600 mg, control | ↑ Lag phase with AT after 4 and 8 weeks compared to control<br>= Maximum rate of propagation |
| Jialal et al. (1995) [60] | Healthy subjects ($n$=48) | Randomized<br>Free-living<br>Supplements provided<br>8 weeks | 60, 200, 400, 800 or 1200 IU, placebo (soybean oil) | ↑ Lag phase with AT (400, 800 and 1200 IU/day) compared to baseline<br>↓ Oxidation rate (CD) with AT (800 and 1200 IU/day) compared to baseline |
| Princen et al. (1995) [59] | Healthy subjects ($n$=20) | Parallel<br>Free-living<br>Supplements provided<br>2 weeks | 25, 50, 100, 200, 400 or 800 IU | ↑ Lag phase with AT (25, 100, 200, 400 and 800 IU/day) compared to baseline<br>↓ Propagation rate with AT (400 and 800 UI/day) compared to previous dose |
| Reaven et al. (1995) [64] | T2D males ($n$=21) | Randomized<br>Free-living<br>Supplements provided<br>10 weeks | 1600 IU or placebo | ↑ Lag phase with AT compared to previous dose |
| Suzukawa et al. (1995) [58] | Healthy subjects ($n$=8) | Free-living<br>Supplements provided<br>4 weeks | 150 mg (1 week) and 300 mg (3 weeks) | ↑ Lag phase with AT compared to baseline<br>↓ Propagation rate compared to baseline |
| Fuller et al. (1996) [65] | Diabetic subjects ($n$=28) | Randomized<br>Free-living<br>Supplements provided<br>8 weeks | 1200 IU or placebo | ↑ Lag phase with AT compared to baseline and to placebo<br>↓ Oxidation rate (CD) with AT compared to baseline |
| Mol et al. (1997) [61] | T2D subjects ($n$=11)<br>Healthy subjects ($n$=14) | Free-living<br>Supplements provided | 600 IU | ↑ Lag phase in three groups compared to baseline |

## FUNCTIONAL INGREDIENTS

### Lipidic Ingredients from vegetable products

Tocopherols

What are they? Structure - types-function

Why functional ingredients? (bioactivity)

Do you have scientific evidence? Articles

Do you have clinical studies? Health claims

↓ **OK, Lets make it**

**Where are they? Sources-industrial production**

**What functional food is the ideal for this ingredient? Available, compatible, complementary**

**Different cases:tocos-sterols-carots-etc**

## *TOCOPHEROLS*

### Where are they? Natural sources

- Found in all photosynthetic organisms
  - always located in membrane of the cell

- Sources in diet:
  - Fats and oils (corn, soybean, palm, cottonseed, sunflower..)
  - Cereal grains (minor)
  - Vegetable products

- Effect of growing conditions
  - Higher levels at higher temperatures

## *TOCOPHEROLS*

### Where are they? Industrial production

- Extraction from natural sources
  - Deodorization distillates from edible oil processing
  - From soybean oil (10-14% tocos) or corn

- Chemical synthesis
  - Synthesis of 8 stereoisomers- high yield---esterification (stable)—purification (vacuum distillation)

# TOCOPHEROLS

## Where are they? Sources-industrial production

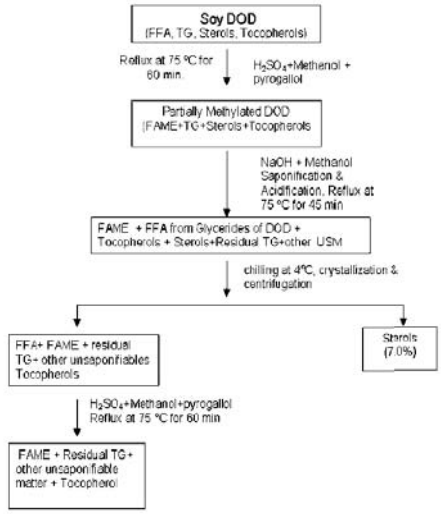



**Fig. 1** Schematic representation of chemical modification process of soy DOD

# TOCOPHEROLS

## Where are they? Sources-industrial production

Eur Food Res Technol (2003) 217:427–433
DOI 10.1007/s00217-003-0781-5

ORIGINAL PAPER

G. K. Nagesha · B. Manohar · K. Udaya Sankar

**Enrichment of tocopherols in modified soy deodorizer distillate using supercritical carbon dioxide extraction**

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**Abstract** Supercritical carbon dioxide ($SC\text{-}CO_2$) extraction of chemically modified Soy Deodorizer Distillate (DOD) at three levels of pressure (180–300 bar) and temperature (40–60 °C) to optimize the conditions for enrichment of tocopherols in the raffinate was carried out. After modification, soy DOD contained about 90% of fatty acid methyl esters (FAME), showed improved solubility in $SC\text{-}CO_2$, and better extraction rate. Since fatty acid methyl esters are more volatile, they are extracted preferentially over tocopherols and other high molecular weight compounds. Higher levels of pressure and temperature resulted in increased solubility of compounds with high molecular weight and tocopherols due to increased density of $SC\text{-}CO_2$. So the extraction at higher pressures and temperatures resulted in a better yield of FAME along with tocopherols in the extract and this in turn decreased the degree of enrichment of tocopherols in the raffinate. However, specific level of pressure and temperature of extraction caused the increase in the solubility of FAME due to their volatility and results in enhanced enrichment of tocopherols in the raffinate. It was observed that the enrichment of tocopherols to ten times the original concentration of feed occurred at extraction pressure of 180 bar and temperature of 60 °C of $SC\text{-}CO_2$.

**Keywords** Soy DOD (deodorizer distillate) · Modified soy DOD · Supercritical carbon dioxide ($SC\text{-}CO_2$) · Enrichment · Tocopherols · Fatty acid methyl esters (FAME)

**Introduction**

Soy deodorizer distillate (DOD) is a valuable by-product in the refining of vegetable oils and amounts to 0.3–0.5% of the feed stock. It is a complex mixture of triglycerides, free fatty acids, sterols, tocopherols, hydrocarbons and other unsaponifiables. Among these tocopherols and sterols find extensive applications in cosmetic, pharmaceutical industries and as natural anti-oxidants in processed foods [1]. Tocopherol, which is physiologically active as vitamin E, is a major natural antioxidant and is used for protection of fats and oils from atmospheric oxidation. Particularly the α-isomer is used as a pharmaceutical substance, an ingredient of cosmetics, and a health food. A mixture of α-, γ-, and δ-isomers containing 60 wt% tocopherols is widely utilized as an additive in processed foods [2]. In the present study the deodorizer distillate which contains 2.9% tocopherol is explored as a principal raw material source for the production of natural tocopherol.

Solvent extraction, chemical treatment, and molecular distillation were employed for recovery of tocopherols and sterols from soybean oil scum by Kim and Rhee [3]. Abu Shuhur and Neeman [4] has tried to separate sterols and tocopherols by the combination of solvent extraction and chemical treatment resulted in poor yields. Due to the similar volatility of sterols, tocopherols, and fatty acids, it is quite difficult to separate sterols and tocopherols from fatty acids during fractional distillation using steam stripping at high vacuum.

Supercritical carbon dioxide ($SC\text{-}CO_2$) extraction is promising as a separation technology and attempts have been made to isolate tocopherols from natural materials by this process. $SC\text{-}CO_2$ has been widely applied as a promising alternative to conventional extraction processes used in the food and pharmaceutical industries. It has some advantages over conventional separation processes. The most important advantage of utilizing $SC\text{-}CO_2$ is the easy separation of the solvent from the extracted material and it operates at ambient temperature that does not affect the heat sensitive compounds. Further, $SC\text{-}CO_2$ provide lower mass transfer resistance than those in conventional separation processes. A number of references dealing with the basic principles and applications of $SC\text{-}CO_2$ are available elsewhere [5, 6, 7].

G. K. Nagesha · B. Manohar · K. Udaya Sankar (✉)
Department of Food Engineering,
Central Food Technological Research Institute,
57 0013 Mysore, India
e-mail: udaya@cftri.res.nic.in

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www.elsevier.com/locate/foodchem

Developing functional foods using red palm olein. IV. Tocopherols and tocotrienols

Jamila M. Al-Saqer[a], Jiwan S. Sidhu[a,*], Suad N. Al-Hooti[a], Hanan A. Al-Amiri[a], Amani Al-Othman[b], Latifa Al-Haji[c], Nissar Ahmed[c], Isa B. Mansour[d], Johari Minal[d]

[a]*Biotechnology Department, Kuwait Institute for Scientific Research, PO Box 24885, 13109 Safat, Kuwait*
[b]*Systems Development Department, Kuwait Institute for Scientific Research, PO Box 24885, 13109 Safat, Kuwait*
[c]*Central Analytical Laboratories, Kuwait Institute for Scientific Research, PO Box 24885, 13109 Safat, Kuwait*
[d]*Malaysian Palm Oil Board, PO Box 10620, 50720 Kuala Lumpur, Malaysia*

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**Abstract**

Two functional foods (pan bread and sugar-snap cookies), were prepared using red palm olein (RPOL) and red palm shortening (RPS) with the objective of providing higher amounts of antioxidant vitamin E in our diet. The total vitamin E contents in the palm oils used in this study were significantly higher (ranging from 717.8 to 817.7 mg/kg fat) than those in the palm shortenings (ranging from 451.0 to 479.9 mg/kg fat). Tocotrienols were found to be the predominant compounds (about 81–84% of the total vitamin E) in all of the palm oil and palm shortening samples. The total tocopherol and total tocotrienol contents were significantly higher in bread made with control shortening (325.0 and 468.0 mg/kg fat) than in that made with a 75% replacement level of RPOL (192 and 407 mg/kg fat) or RPS (101.4 and 300 mg/kg fat). Similar trends were observed in the white bread and the brown bread made with RPOL and RPS. The tocotrienol contents in cookies made with varying levels of replacement RPS ranged from 229.1 to 317.8 mg/kg fat, compared with 379.4 to 672.3 mg/kg fat in cookies made with RPOL. The tocotrienols were found to be the predominant fraction in cookies made either with RPS (58 to 68%) or with RPOL (about 69 to 83% of the total vitamin E). Cookies, being higher in fat contents, would be better providers of these desirable phytochemicals and antioxidant vitamins than breads.
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*Keywords:* Functional foods; Red palm olein; Vitamin E; Tocopherols; Tocotrienols; Bread; Cookies; Nutritive value

## What functional food is the ideal for this ingredient?

**CONCLUSION: Functional food ingredient?**

- **Any food with lipids**
  - **Especially, food with PUFA**
    - **New enriched omega-3 food**

- **Combination with other antioxidants**
    - **Synergistic effects**

- **No regulation problem, natural and frequent ingredient**

- **Important to combine with other active components in new functional foods**



**CONCLUSION:**

**VITAMIN E…**

**…Functional food ingredient?**

## Lipidic Ingredients from vegetable products

Tocopherols **Sterols** Carotenoids

Other antioxidants

What are they? Structure - types-function

Why functional ingredients? (bioactivity)

Do you have scientific evidence? Articles

Do you have clinical studies? Health claims

↓ **OK, Lets do it**

Where are they? Sources-industrial production

What functional food is the ideal for this ingredient?
Available, compatible, complementary

**Produce and sell it !**

PHYTOSTEROLS…..

STEROLS
STANOLS
STEROL ESTERS
STANOL ESTERS

…added EVERYWHERE

## STEROLS

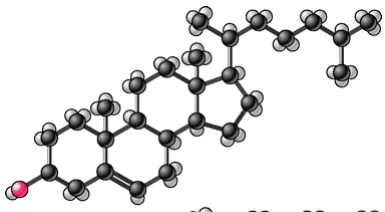
### What are they? Structure - classes

- Sterols and Stanols
- Sterol esters and stanol Esters
- Minor components in oils (unsaponifiable)
- Both tocopherols and carotenoids are groups of well known compounds with interesting activity
- Very common, frequent in small amounts
- Not clear beneficial activity

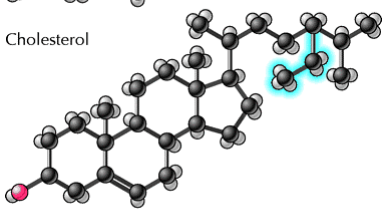
**Cholesterol** ⟷ **Phytosterols**

## STEROLS

### What are they? Structure - classes



Cholesterol



β-Sitosterol

## STEROLS

### What are they? Structure – types-function

- Structure: Sterols and Stanols
- Phytosterols: Sterol and stanol Esters
- Main activity: Inhibit cholesterol absorption
  - Compete in micelles for absorption of lipids with cholesterol
- Natural, mostly sterols
- STANOL : no absorption
- Diverse family – similar behaviour
- Industrial sources:
  - tall oil
  - deodorizer oil distillates
- Hydrogenated to produce stanols

## STEROLS

### What are they? Structure – types-function

- Structure: Sterols and Stanols
- Sterol and stanol Esters

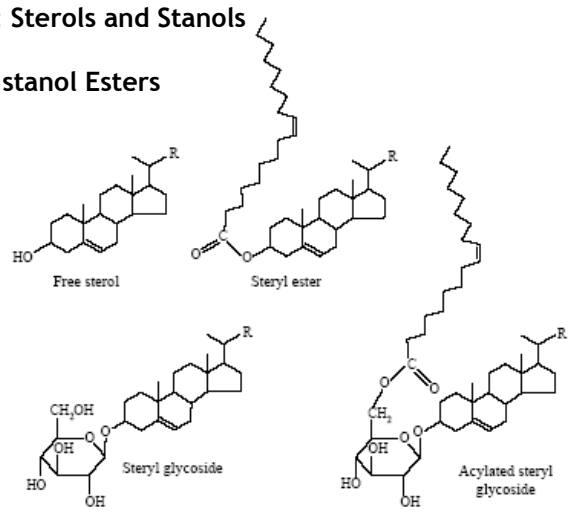


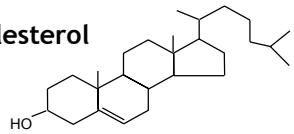

FIGURE 1. Examples of basic structures of free sterol and steryl conjugates. R (side chain) varies between different sterols.

## STEROLS

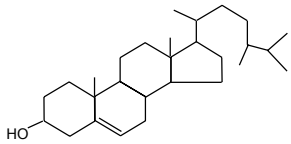
### What are they? Structure – types-function

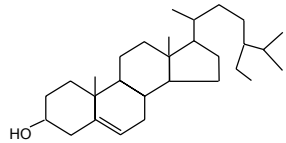
**Sterols and Stanols**

**cholesterol**

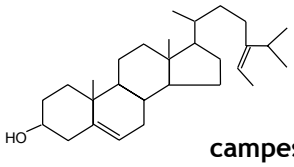



**D-5-avenasterol**




**beta-sitosterol**



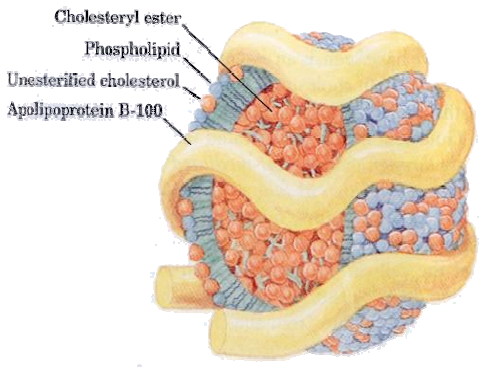

**campesterol**

## STEROLS

### Why functional ingredients? (bioactivity)

- **Main biological effect: competition in micelles**

- **Other demonstrated effects:**
  **LDL-c**



- Phytosterols:
  - Sterols and Stanols
  - Sterol Esters and stanol Esters

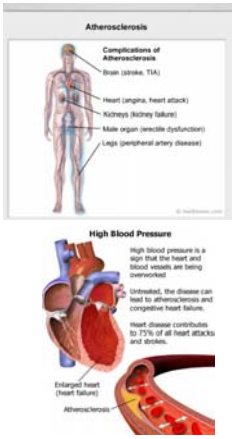
•Main activity:
•Reduce the absorption of cholesterol from the gut and so lower serum concentrations of cholesterol

•Plant sterols or stanols that have been esterified to increase their lipid solubility can be incorporated into foods



# Atherosclerosis

- hardening of the arteries due to the deposition of atheromas
- heart disease is the leading cause of death
- caused by the deposition of cholesteryl esters on the walls of arteries
- atherosclerosis is correlated with high LDL and low HDL



- Sterols Hydrogenated to produce stanols
- Free Sterols Esterified to produce sterol esters (stanol esters)

## • Sterol Esters and stanol Esters

- Plant sterols or stanols that have been esterified to increase their lipid solubility can be incorporated into foods

- Diverse family – similar behaviour

**Main activity:**
**Reduce the absorption of cholesterol from the gut and so lower serum concentrations of cholesterol**



- Structure: Sterols and Stanols

- Sterol and Stanol Esters

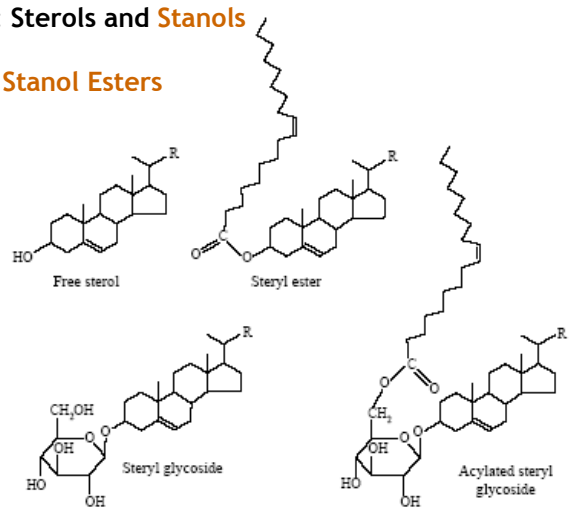



FIGURE 1. Examples of basic structures of free sterol and steryl conjugates. R (side chain) varies between different sterols.

## *STEROLS*

Is there any scientific evidence? Articles

- **Chemical characterization and Different tests (activity *in-vitro*…): many**
- **Epidemiological studies: several evidences**
- **Animal model tests (*in-vivo)***
- **Pre-clinical:Dose effects, toxicological risks, etc.: studied**
- **Clinical studies, Intervention studies: many**

Stanols and sterols have been well tolerated in numerous clinical trials on over 1800 people, with doses up to 25 g per day.

Studies have established that at least 1 gram of stanol and sterol in ester form can result in a statistically significant reduction in cholesterol.

Recent studies of plant sterol and stanol esters in humans have shown, however, that maximum cholesterol-lowering benefits are achieved at doses of 2-3 g per day.

## *STEROLS*

Is there any scientific evidence? Articles

- **Chemical characterization and Different tests (activity *in-vitro*…): many**
- **Epidemiological studies: several evidences**
- **Animal model tests (*in-vivo)***
- **Pre-clinical:Dose effects, toxicological risks, etc.: studied**
- **Clinical studies, Intervention studies: many**

- **Reliability: Total**
- **Controversial: very little for a new product**
  - **None on effect**
  - **Some on high dose supplementation effect**
  - **Much on interaction with fat-soluble vitamins and carotenoids**
- **Accepted: completely**
- **Approved health claims: yes, Ok (US, UE, etc.)**

## STEROLS

### Where are they? Sources

- Phytosterols:
  - Sterols and Stanols
  - Sterol Esters and stanol Esters
- Industrial sources:
  - tall oil
  - deodorizer oil distillates

- Hydrogenated to produce stanols

- **Sterol Esters and stanol Esters**

- Plant sterols or stanols that have been esterified to increase their lipid solubility can be incorporated into foods

## STEROLS

### Are ther clinical studies? Health claims

**Properties:** (When at least 1 gram per day is consumed in ester form for statistically significant effects)

- Reduce total cholesterol
- Reduce LDL cholesterol
- Do not affect HDL cholesterol or triglycerides

"Foods that may qualify for the health claim based on plant sterol ester content include spreads and salad dressings. Among the foods that may qualify for claims based on plant stanol ester content are spreads, salad dressings, snack bars, and dietary supplements in softgel form. Foods that carry the claim must also meet the requirements for low saturated fat and low cholesterol, and must also contain no more than 13 grams of total fat per serving and per 50 grams." (FDA, 2000)

**Health claims:**
Diets low in saturated fat and cholesterol that include at least 1.3 grams of plant sterol esters or 3.4 grams of plant stanol esters, consumed in 2 meals with other foods, may reduce the risk of heart disease. In addition to and in the presence of the health claim, products with plant stanol/sterol esters may also state that they can reduce cholesterol levels.

Plant sterols first came on the European market in 1995 in a fortified margarine called Benecol, launched in Finland by Raisio Life Sciences. But even after hitting US shores in 1999 by McNeil (Benecol) and Unilever (Take Control), sales did not live up to expectations. The cholesterol-lowering potential of stanols and sterols had been known for 50 years, and with an estimated 105 million Americans with borderline or high cholesterol, the market potential certainly was there. What happened?

The trouble was, margarines are not commonly associated in consumers' minds with healthful eating, and in America at least, spreads are not routinely found on the daily dinner table.

In 2002, the Food & Drug Administration finally allowed the cholesterol-lowering heart-health claim for plant sterols to be available to a wider range of foods, and if the flurry of product launches of the past few years is any indication, the train has finally taken off.



- **Accepted health claims: Now in a second phase:**
- **LONG TERM STUDIES**
- **Possible problems for very large daily ingestion**
- **Interactions:**

**M. Richelle, M. Enslen, C. Hager, M. Groux, I. Tavazzi, J.-P. Godin, A. Berger, S. Metairon, S. Quaile, C. Piguet-Welsch, L. Sagalowicz, H. Green, and L. B. Fay**
**Both free and esterified plant sterols reduce cholesterol absorption and the bioavailability of {beta}-carotene and {alpha}-tocopherol in normocholesterolemic humans**
**Am. J. Clinical Nutrition, July 1, 2004; 80(1): 171 - 177.**

**Noakes, M., Clifton, P., Ntanios, F., Shrapnel, W., Record, I. & McInerney, J. (2002)**
**An increase in dietary carotenoids when consuming plant sterols or stanols is effective in maintaining plasma carotenoid concentrations. Am. J. Clin. Nutr. p.79**

## *STEROLS*

PHYTOSTEROLS…..

STEROLS
STANOLS
STEROL ESTERS
STANOL ESTERS

…added EVERYWHERE

## *FUNCTIONAL INGREDIENTS*

### Lipidic Ingredients from vegetable products

Tocopherols Sterols Carotenoids

What are they? Structure - types-function

Why functional ingredients? (bioactivity)

Do you have scientific evidence? Articles

Do you have clinical studies? Health claims

↓ OK, Lets do it

Where are they? Sources-industrial production

What functional food is the ideal for this ingredient?
Available, compatible, complementary

**Produce and sell it !**

## FUNCTIONAL INGREDIENTS

**Different cases:tocos-sterols-carots-etc**

Tocoferoles Carotenoides Esteroles

Complexity: Sterols (like one)-Tocos (4+4)-Carotenoids (100s)

Level of scientific evidence? Articles-controversy

Status of clinical studies? Health claims posed

Sources-industrial production:
Tall oil (sterols) DOD (sterols+toco) Plants and algae (carotenoids)

**What functional food is the ideal for this ingredient?**
**Available, compatible, complementary**
**Different cases:tocos-sterols-carots-etc**

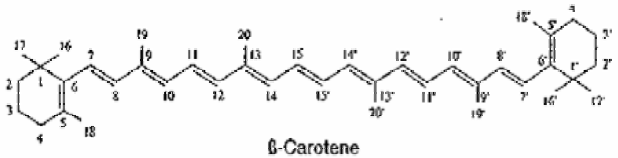
## CAROTENOIDS

**CAROTENOIDS…**

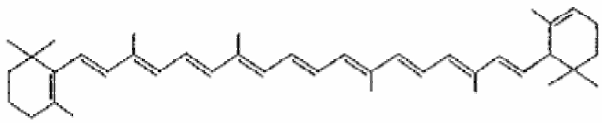
**……NATURAL DIVERSITY**

**…COMPLEXITY OF EFFECTS**

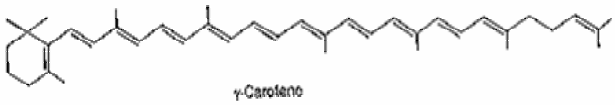
- **Structure**



ß-Carotene



α-Carotene

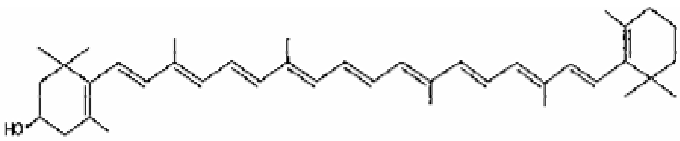


γ-Carotene

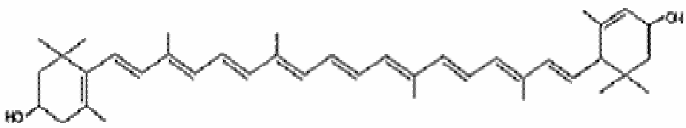
CAROTENOIDS
What are they? Structure – types-function

- **Structure**

Lycopene




ß-Cryptoxanthin




Lutein

## CAROTENOIDS

### Where are they? Sources-industrial production

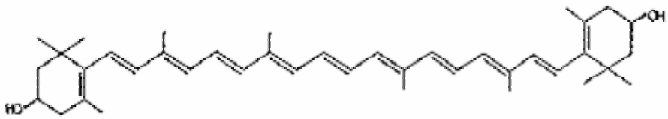
- **Vegetable products**

- Natural Mixed Carotenoids
  family of naturally occurring yellow, orange and red pigments
  found in various fruits, cruciferous, yellow and dark - green vegetables and also abundantly in algae.

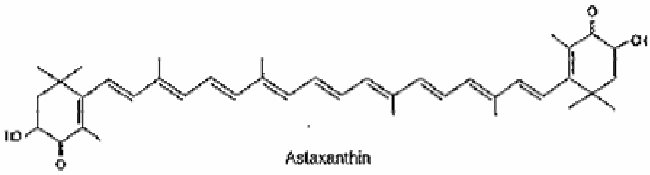
## CAROTENOIDS

### What are they? Structure – types-function

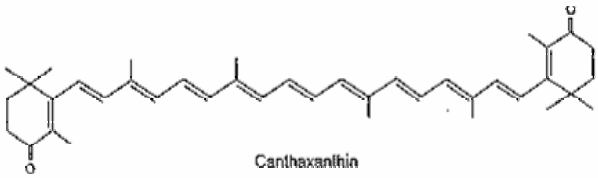
- **Structure**




Zeaxanthin

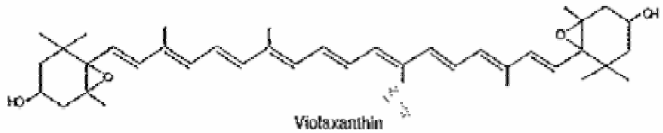



Astaxanthin

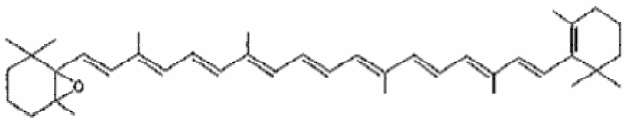



Canthaxanthin

- **Structure**




Violaxanthin

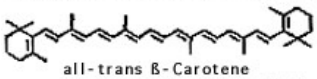



ß-Carotene-5,6-epoxide

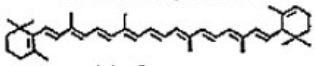
CAROTENOIDS
What are they? Structure – types-function

- **Structure: Carotenes and xanthophylls**

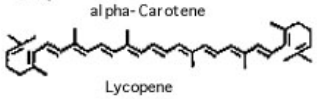
CAROTENES = HYDROCARBONS



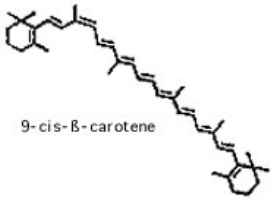
all-trans ß-Carotene



alpha-Carotene

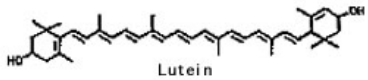


Lycopene

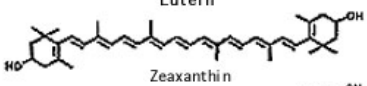


9-cis-ß-carotene

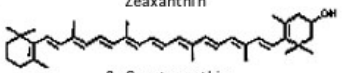
XANTHOPHYLLS = OXYCAROTENOIDS



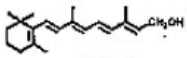

Lutein



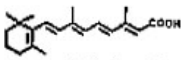

Zeaxanthin




ß-Cryptoxanthin




Retinol



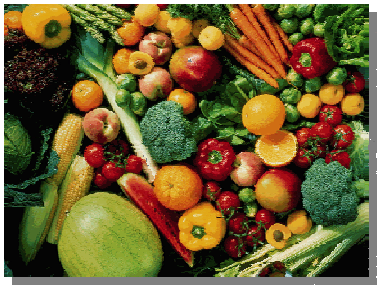

Retinoic acid

## CAROTENOIDS

### What are they? Structure – types-function

**Activity: Carotenes and xanthophylls**

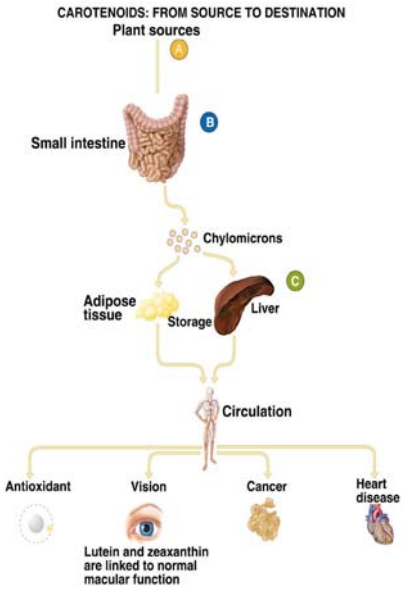
- Main activity: antioxidant
- Pro - Vitamin A
- Natural (some synthetic)
- Diverse and complex family



## CAROTENOIDS

### Why functional ingredients? (bioactivity)

- Main biological effect
  - Some pro.Vitamin A
  - Mostly antioxidants
- Other demonstrated effect
  - Cancer prevention



## Why functional ingredients? (bioactivity)

- **Main biological effect:**
- **Other demonstrated effect:**

- Additional physiologic effects beyond vitamin A
  - Serving as an "antioxidant"
    - Remove excess "electrons" from cell system
    - Electrons (free radicals) damage cells and DNA
      - Can cause mutations
  - Protecting from cancer (related to antioxidant function?)
  - Protecting from heart disease?
  - Supports immune system function



## Carotenoids. Roles in the Body

- Promote vision (retinal)
  - Maintenance of the cornea
  - Converting light energy into nerve impulses
- Participate in protein synthesis and cell differentiation
  - Differentiation of epithelial and goblet cells (retinoic acid)
- Support reproduction and growth
- Support immunity (retinoic acid and carotenoids)
- Involved in bone growth and remodeling
- Synthesis of glycoproteins
- Antioxidant activity (ß-carotene….)

## CAROTENOIDS

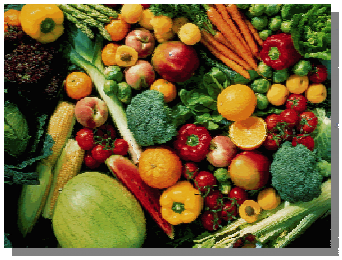
### Do you have clinical studies? Health claims

- Pre-clinical for some of them
- Different tests (in-vitro, in-vivo…)
  - Agreement on antioxidant activity
- Epidemiological studies form natural mixtures of carotenoids
- Clinical studies:
- Controversial: Strong
  - Problem with beta-carotene and smokers
- Enrichment Accepted: ??
- Accepted health claims:

## CAROTENOIDS

### Where are they? Sources

Sources: Carotenes and xanthophylls



- Natural (some synthetic)
- Diverse and complex family
- Industrial sources:

  biotechnological production with yeasts/fermentation

  microalgae (Dunaliella, Haematoccocus, etc.)

  chemically synthesized

## FUNCTIONAL INGREDIENTS

**Different cases:tocos-sterols-carots-etc**

Tocoferoles | Carotenoides | Esteroles

Complexity: Sterols (like one)-Tocos (4+4)-Carotenoids (100s)

Level of scientific evidence? Articles-controversy

Status of clinical studies? Health claims posed

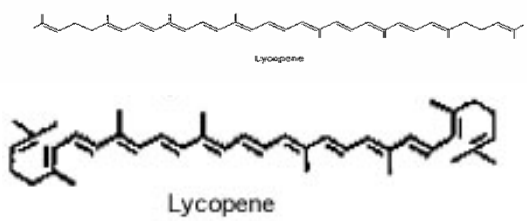
Sources-industrial production:
Tall oil (sterols) DOD (sterols+toco) Plants and algae (carotenoids)

**What functional food is the ideal for this ingredient?**
**Available, compatible, complementary**
**Different cases:tocos-sterols-carots-etc**

## CAROTENOIDS

Lycopene from tomato

- Structure of lycopene



## CAROTENOIDS

### Lycopene from tomato

Lycopene

- Biological activity
  - Antioxidant
  - Efficient oxygen quencher and trap peroxyl radicals

- Fresh tomatoes:
  - 80-90% of total carotenoids
  - all-trans-lycopene
  - 0,7-20 mg/100g Wet basis

- Processed tomatoes: increased cis-lycopene (4-27%)

- Human serum: > 50% cis-lycopene

- Skin and pericarp rich in lycopene ---industrial waste material
  *(Al-Wandawi, 1985)*

## CAROTENOIDS

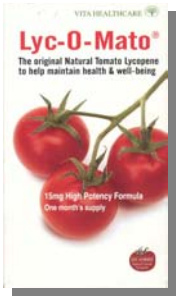
### Lycopene from tomato

Lycopene

- Biological activity
  - Antioxidant
  - Protection of DN..

- Lycopene content affected by agricultural practices:
  - *irrigation, fertilization, temperature and light, variety, degree of maturity at harvest, harvest time, postharvest storage (Shi, 2002)*

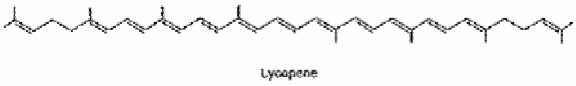
- Bioavaliability for humans:
  - Isomeric form
  - food matrix
  - lipid content
  - other interactions (fiber)



## CAROTENOIDS

### Lycopene from tomato

- Biological activity
  - Antioxidant




- Health promoting effect
  - Protection against some cancers:
    - Prostate, breast, digestive tract, epithelial… (Colditz, 1985, Gerster,1997)
  - Studies (90`s) on tomato consumption and protection against digestive cancer (Franchesi, 1994,
  - Giovannucci, 1995, prostate cancer in 48,000 men / 4 years / 46 fruits&vegetables
    - lower risk strongly associated with tomato sauce comsumption
  - Atherosclerosis and Coronary Heart Disease
    - Reduce LDL oxidation (Kristenson, 1997)
- Recomendación diaria 10-50 mg

## CAROTENOIDS

### Lycopene from tomato

**Roche Lycopene Self-Affirmed GRAS**

*PARSIPPANY, N.J.*—Roche Vitamins Inc. announced it has self-affirmed its l
GRAS (Generally Recognized as Safe) through an independent panel evaluation
been scientifically linked to reducing the risk of certain cancers, as well as impro
lung, cardiovascular and stomach health, according to the company. Roche lyco
in both water-dispersible and oil-soluble forms and is suited for an array of proc
nutritional bars, beverages and dairy products. Roche (www.roche.com) noted
stable, easy to process and will not affect the taste profile of food and beverage

LycoRed
Natural Products Industries Ltd.

**Mission Statement**
LycoRed Natural Products Industries Ltd., is a research and development company dedicated to the scientific and commercial development, production and marketing of natural products based on the phytonutrients from tomato, including the carotenoids lycopene, beta-carotene, phytoene and phytofluene, phytosterols and other important phytochemicals.

**About the Company**
LycoRed is a subsidiary of Makhteshim Agan Industries, Ltd., one of Israel's largest industrial groups, a public company, with a turnover in 2001 of more than $900 million. LycoRed supervises production of **LYC-O-MATO®** tomato extract from the field to the finished product. Coordination of the commercial growing of a non-genetically modified, lycopene-rich variety of tomato plant is just the start of the multi-stepped process that assures high product quality. Phytochemicals are extracted using proprietary processing technology. Specialized extraction, handling, packing and storing techniques assure a long shelf life.

LycoRed markets **LYC-O-MATO®**, its scientifically standardized, natural tomato extract, worldwide.

LycoRed's **LYC-O-MATO®** formulations have defined functional properties supported by basic research and clinical research. A growing body of scientific evidence

## CAROTENOIDS

### Lycopene from tomato

Lycopene

- Extraction of lycopene from tomatoes
- Need of high purity lycopene products –
  - food safety regulations
- Need of further studies on toxicology and bioavailability
- Large amount of skin and pericarp discarded waste of peeling

- Processed tomatoes: increased cis-lycopene (4-27%)
- New extraction technologies under development
  - complex bioactive components
  - sensitive to oxygen, thermal, (oxidation, isomerization, etc.)

## CAROTENOIDS

### Lycopene from tomato waste

Limitation: reliable Industrial source

Difficult extraction

2638 J. Agric. Food Chem. 2002, 50, 2638−2643

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

**Supercritical Fluid Extraction of Lycopene from Tomato Processing Byproducts**

N. L. Rozzi,[†] R. K. Singh,[‡] R. A. Vierling,[§] and B. A. Watkins*,[†]

Center for Enhancing Foods to Protect Health, Department of Food Science, and Department of Agronomy, Purdue University, West Lafayette, Indiana 47907

Tomato seeds and skins acquired from the byproduct of a local tomato processing facility were studied for supercritical fluid extraction (SFE) of phytochemicals. The extracts were analyzed for lycopene, β-carotene, α-carotene, α-tocopherol, γ-tocopherol, and δ-tocopherol content using high-performance liquid chromatography−electrochemical detection and compared to a chemically extracted control. SFEs were carried out using $CO_2$ at seven temperatures (32−86 °C) and six pressures (13.78−48.26 MPa). The effect of $CO_2$ flow rate and volume also was investigated. The results indicated that the percentage of lycopene extracted increased with elevated temperature and pressure until a maximum recovery of 38.8% was reached at 86 °C and 34.47 MPa, after which the amount of lycopene extracted decreased. Conditions for the optimum extraction of lycopene from 3 g of raw material were determined to be 86 °C, 34.47 MPa, and 500 mL of $CO_2$ at a flow rate of 2.5 mL/min. These conditions resulted in the extraction of 61.0% of the lycopene (7.19 μg lycopene/g).

KEYWORDS: Lycopene; supercritical fluid extraction; carotenoids; tocopherols; chromatography

**INTRODUCTION**

Lycopene is an acyclic carotenoid containing 13 double bonds and is chiefly found in tomatoes, where it provides their rich, red color. Other sources of lycopene include apricots, pink grapefruit, guava, and watermelon (*1*). Although there are other products that contain lycopene, tomatoes are the main source. Sharma and Le Maguer (*2*) evaluated the nutrient content of tomato juice fractions and determined that 72−92% of the lycopene in a tomato is found in the water insoluble fraction

nucci and Clinton (*8*) reported that epidemiological data supported these claims. In addition, Rao and Agarwal (*9*), Weisburger (*10*), Clinton (*11*), Astorg (*12*), and Gerster (*13*), examined the health benefits of lycopene and correlated the consumption of food containing lycopene to the prevention of several cancers.

Supercritical fluid $CO_2$ has been used to extract α- and β-carotene and lycopene from several plant sources (*14*−*17*). Marsili and Callahan (*14*) compared supercritical fluid extraction

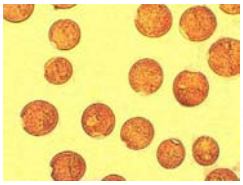
- **Not synthetic—not single carotenoid:**
- Natural Mixed Carotenoids

- **Carrots, corn, colored vegetables….**

- **No industrial scale extraction**

- **Microalgae are a new source of carotenoids and other bioactive ingredients**
  - **Chlorella,**
  - **Spirulina,**
  - **Dunaliella,**
  - **Microalgae Haematococcus pluvialis,**
  - **etc.**

## WHY TO USE ALGAE?



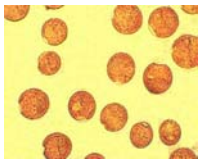
Man has been using algae since long time ago (thousand years) with different purposes.

In China they have been used since 2700 a.c.; greeks and romans used algae as food, as medicinal plants and cosmetics.

Aztecs used Spirulina, harvested from Texcoco Lake, as proteic supplement.

In the world, around 7 billion Tons of algae (net weigth) are processed, being asian countries the main producers (around 80% of the raw material).

# WHY TO STUDY *MICROALGAE?*

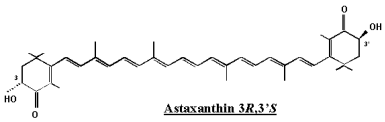


- The photosynthetic activity of the algae generates O2, to avoid attack of O2 the microalgae accumulate antioxidant compounds
- Some compounds with antiviral and antimicrobial activity have been described
- At present, research is conducted to verify the efficiency of algae against certain diseases and illness: trombosis, cholesterol, rheumatism
- The main consideration is safety-toxicity, any possibility of toxicity precludes the use of the microalgae.
- The literature shows that ß-carotene and PUFAS have been widely studies, while other compounds with potential activities (antioxidant, antimicrobial or antiviral (polyphenols, peptides, polysaccharides)) have not been studied yet.
- Commercial availability



- **Microalgae are a new source of carotenoids and other bioactive ingredients** Chlorella, Spirulina, Dunaliella, etc.

- **Biological activity**
  - **Antioxidant**
  - **Efficient oxygen quencher**
  - **trap peroxyl radicals**

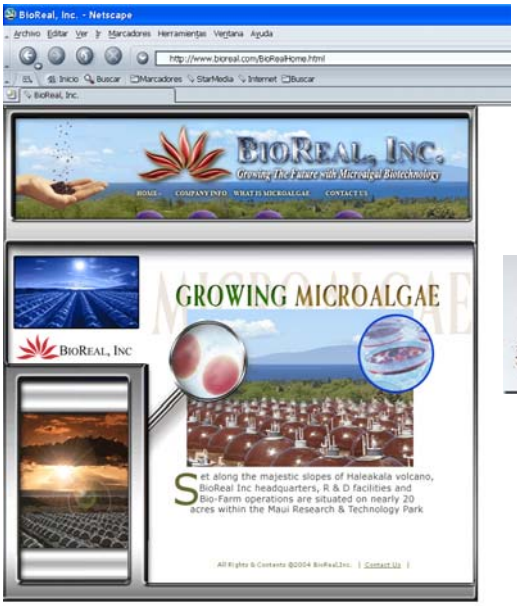



Astaxanthin 3*R*,3'*S*

- **Microalgae Haematococcus pluvialis:**
-

## CAROTENOIDS

•Extraction of astaxanthin from Haematococcus



## CAROTENOIDS

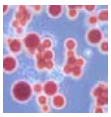
•Extraction of astaxanthin from Haematococcus

## CAROTENOIDS

•Extraction of astaxanthin from Haematococcus

Antioxidant more powerful than other carotenoids
Benefits for carciovascular health
for macular degeneration (**eye health**)

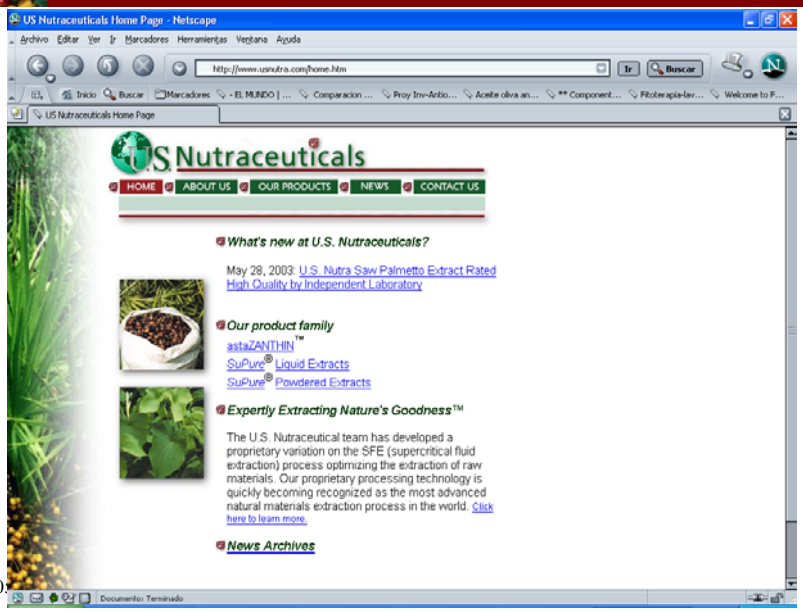
Haematoccocus pluvialis
(Empresas: US Nutra (LaHaye), Separex, Fuji, Cyanotech…)



" As an antioxidant, astaxanthin has
been shown to be up to 500 times more powerful than Vitamin E
and more than 10 times as potent as beta-carotene."

## CAROTENOIDS

•Extraction of astaxanthin from Haematococcus

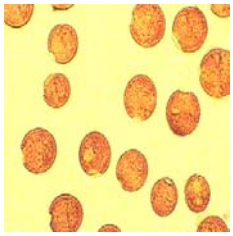


## CAROTENOIDS

•Extraction of astaxanthin from Haematococcus

## SELECTED MICROALGAE with CAROTENOIDS

Spirulina platensis



Dunaliella salina

I. Non toxic (some of them have been used as food).

II. Industrial production (commercially available).

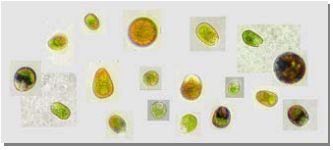
III. Complementary composition (*Dunaliella* rich in ß-carotene, *Spirulina* is rich in different pigments).

## CAROTENOIDS

### Beta-carotene from microalgae: Dunaliella

•Microalgae are a new source of carotenoids and other bioactive ingredients **Chlorella, Spirulina, Dunaliella, etc.**

- Biological activity
  - •Antioxidant
  - •Efficient oxygen quencher
  - •trap peroxyl radicals



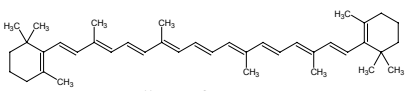
**•Microalgae Dunaliella salina**

Is a single celled, salt-water micro-algae accumulates massive amounts of carotenoids under appropriate growth conditions.

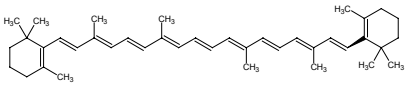
Natural Mixed Carotenoids found in Dunaliella salina are among nature's best antioxidants, containing a variety of carotenoids including β-Carotene, α-carotene and Xanthophylls like Zeaxanthin, Cryptoxanthin and Lutein.

## CAROTENOIDS

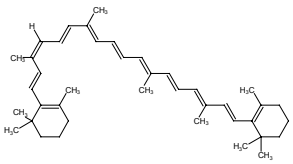
### Functional Compounds from *Dunaliella salina*:(SFE, ASE)



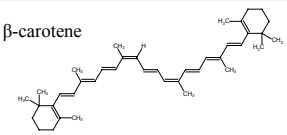
all-trans β-carotene



α-carotene



9-cis β-carotene



13-cis β-carotene

15-cis β-carotene

- Composition related to different activity of β-carotene as antioxidant
- Higher antioxidant activity of 9-cis isomer in vitro (Levin and Mokady, Free Radical Biology and Medicine, 17, 77-82 (1994))
- Better activity of *Dunaliella* extracts vs synthetic β-carotene in protecting hepathic diseases in vivo

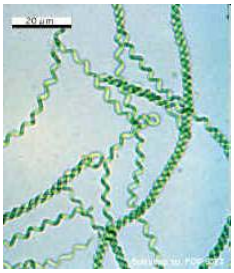
## *CAROTENOIDS*

### Functional Compounds from ***Dunaliella salina***:(SFE, ASE)

- High ratios 9 cis/ all trans β-carotene isomers could be obtained tuning the SFE conditions- studies about the relationship with antioxidant activity are being conducted in our laboratory via in vitro assays
- High yield were obtained by SFE of *Dunaliella salina* microalgae (5-10% for CO2 or CO2 + oil)
- Antimicrobial activity was obtained for PLE extracts of *Dunaliella salina*
- PLE of Dunaliella with ethanol provide good antimicrobial extracts using a green solvent
- The compounds associated to the antimicrobial activity were mainly fatty acids and ionones coming from carotenoid decomposition

## *ANTIOXIDANTS*

### Antioxidants from *Spirulina platensis* (SFE, SWE)




- Blue-Green microalga belonging Cyanobacterium
- Non toxic / Edible
- Live in high salinity lakes
- Up to 60 -70 % dry weight is protein
- Phycocyanins are the main proteins: several functional activities.
- Relatively high content GLA
- Chlorophyll a, xantophylls and other carotenoids are their main photosynthetic pigments

- **Antioxidant capacity of carotenoids**

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

*J. Agric. Food Chem.* **2002,** *50,* 221–226 **221**

**Trolox Equivalent Antioxidant Capacity of Different Geometrical Isomers of α-Carotene, β-Carotene, Lycopene, and Zeaxanthin**

VOLKER BOHM,*,†,‡ NI LUH PUSPITASARI-NIENABER,‡ MARIO G. FERRUZZI,‡ AND STEVEN J. SCHWARTZ‡

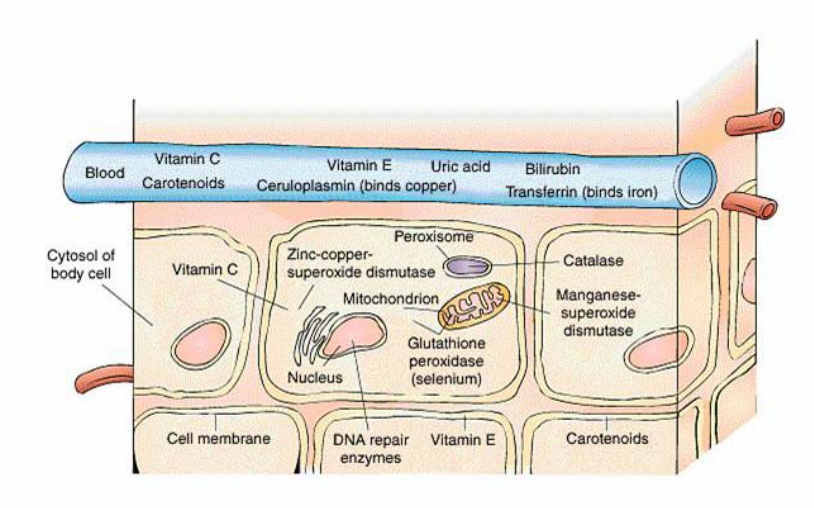
Institute of Nutrition, Friedrich Schiller University Jena, Dornburger Strasse 25-29, 07743 Jena, Germany, and Department of Food Science and Technology, The Ohio State University, 2001 Fyffe Court, Columbus, Ohio 43210-1097

Isomerization of carotenoids, which is often encountered in food processing under the influence of temperature and light, may play a role in the observed protective effects of this group of secondary plant products. Investigation of in vitro antioxidant activity of prominent carotenoid geometrical isomers was undertaken in light of recent reports illustrating a large percentage of carotenoid (*Z*)-isomers in biological fluids and tissues. α-Carotene, β-carotene, lycopene, and zeaxanthin were isolated from foods or supplements and subsequently photoisomerized with iodine as a catalyst. Major *Z*-isomers of each carotenoid were fractionated by semipreparative C-- HPLC. In vitro antioxidant activity of all

To date, published results for the antioxidant activity of β-carotene isomers differ widely due to the various test systems employed.



- **Antioxidant balance**



## Lipidic Ingredients from vegetable products

Tocopherols **Sterols** Carotenoids

Other antioxidants

What are they? Structure - types-function

Why functional ingredients? (bioactivity)

Do you have scientific evidence? Articles

Do you have clinical studies? Health claims

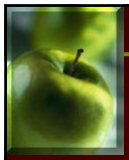
↓ **OK, Lets do it**

Where are they? Sources-industrial production

What functional food is the ideal for this ingredient? Available, compatible, complementary

**Produce and sell it !**





## ANTIOXIDANTS

**...DO WE NEED NEW NATURAL ANTIOXIDANTS ??**

## Antioxidants in food

ANTIOXIDANTS
What are they? Structure – types-function

## Free Radical Damage

- Cancer: prostate, cervical, breast, lung
- Heart disease
- Immune system degeneration
- Stroke, cerebrovascular disease
- Respiratory function
- Cataracts, age-related macular degeneration
- Memory and cognitive function
- Sun-damaged skin, wrinkles
- Diabetes complications

→ Oxidative stress

## Free Radical Damage

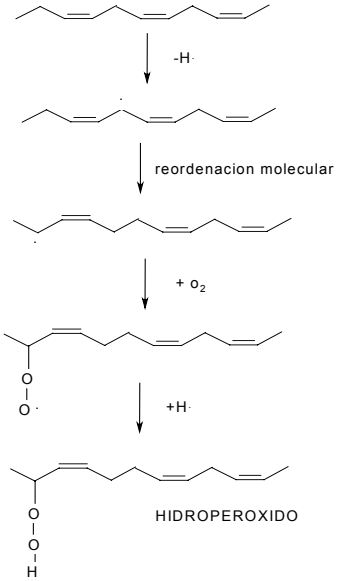
### Where Do Free Radicals Come From?

They form and accumulate in our bodies as a result of modern life:

- Cigarette smoke
- Air pollutants, such as ozone, nitrogen dioxide
- Some food preservatives, including nitrites
- Aerobic activity
- Metabolism of fats



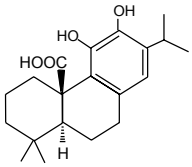
## Free Radical Damage



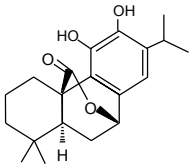
- Phenolic diterpenes: only present in Rosemary (*Rosmarinus officinalis*) and Sage (*Salvia officinalis*)
  - Native from Mediterranean area and South America
- Main component in Rosemary and Salvia: carnosic acid
- Antiox. activity depends on:
  - season, climatic conditions, light, harvesting, storage, etc.
  - Extraction: best results with supercritical CO2

ANTIOXIDANTS
Antioxidant potency of Rosemary diterpenes

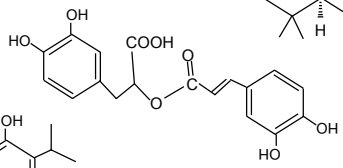
carnosic acid



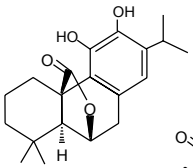

carnosol



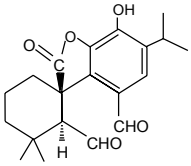

rosmarinic acid




rosmanol



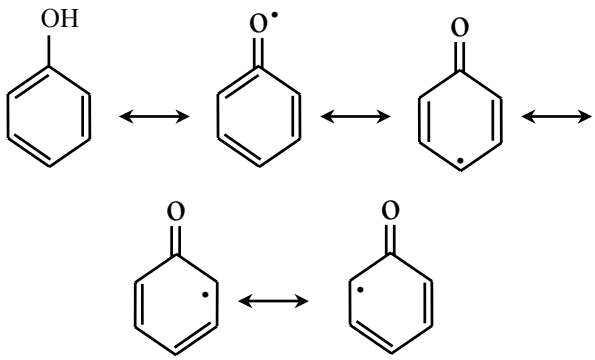

rosmadial




(*Cuvelier et al., 1996*)

## Primary antioxidants

$$ROO + AH = ROOH + A$$






- Rosemary (*Rosmarinus officinalis*) Phenolic diterpenes
- Extraction of carnosic acid with supercritical CO2

- Main component in Rosemary and Salvia
- Commercial extracts
- Clinical studies: Not yet

- Accepted health claims: Not yet
- Functional food: in market

- Rosemary (*Rosmarinus officinalis*) Phenolic diterpenes
- Extraction of carnosic acid with supercritical CO2

European Food Research and Technology
DOI 10.1007/s00217-005-1139-y

Original Paper

## In vitro antioxidant analysis of supercritical fluid extracts from rosemary (*Rosmarinus officinalis* L.)

Sofia Cavero · Laura Jaime · Pedro J. Martín-Álvarez · F. Javier Señoráns · Guillermo Reglero · Elena Ibañez (✉)

S. Cavero · L. Jaime · F. J. Señoráns · G. Reglero
Área de Tecnología de Alimentos, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049 Cantoblanco, Madrid, Spain

P. J. Martín-Álvarez · E. Ibañez
Departamento de Caracterización de Alimentos, Instituto de Fermentaciones Industriales (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain

- Rosemary (*Rosmarinus officinalis*) Phenolic diterpenes
- Extraction of carnosic acid with subcritical water

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

*J. Agric. Food Chem.* **2003**, *51*, 375–382 **375**

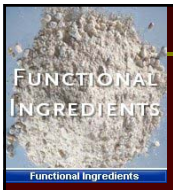
## Subcritical Water Extraction of Antioxidant Compounds from Rosemary Plants

Elena Ibañez,*,† Alena Kubátová,‡ F. Javier Señoráns,§ Sofia Cavero,§ Guillermo Reglero,§ and Steven B. Hawthorne‡

Instituto de Fermentaciones Industriales, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain, Energy and Environmental Research Center, University of North Dakota, Campus Box 9018, Grand Forks, North Dakota 58202, and Área de Tecnología de Alimentos, Universidad Autónoma de Madrid, Ciudad Universitaria de Cantoblanco, 28049 Madrid, Spain

Subcritical water extraction at several temperatures ranging from 25 to 200 °C has been studied to selectively extract antioxidant compounds from rosemary leaves. An exhaustive characterization of the fractions obtained using subcritical water at different temperatures has been carried out by LC-MS, and the antioxidant activities of the extracts have been measured by a free radical method (DPPH). Results indicate high selectivity of the subcritical water toward the most active compounds of rosemary such as carnosol, rosmanol, carnosic acid, methyl carnosate, and some flavonoids such as cirsimaritin and genkwanin. The antioxidant activity of the fractions obtained by extraction at different water temperatures was very high, with values around 11.3 μg/mL, comparable to those achieved by SFE of rosemary leaves. A study of the effect of the temperature on the extraction efficiency of the most typical rosemary antioxidant compounds has been performed.

KEYWORDS: Antioxidant activity; antioxidant compounds; rosemary plants; SFE; subcritical water extraction




## *FUNCTIONAL INGREDIENTS*

## *FUNCTIONAL FOODS*

### Lipophilic Ingredients from vegetable products

**Bioactive and health promoting ingredients from vegetable products**

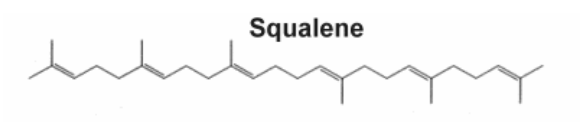
**Potential functional food ingredients**

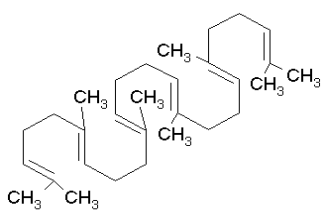
## Bioactive and health promoting ingredients from vegetable products

1. FATTY ACIDS (OLEIC, LINOLENIC, OMEGA-3, ETC.)
2. TOCOPHEROLS
3. STEROLS
4. CAROTENOIDS
5. OTHER ANTIOXIDANTS: PHENOLIC DITERPENES
6. ISOFLAVONES AND ISOFLAVONOIDS
7. FLAVONOIDS
8. PHENOLIC COMPOUNDS
9. ORGANOSULFUR COMPOUNDS
10. ETC…



## Other Candidates:

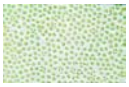


## OPPORTUNITIES

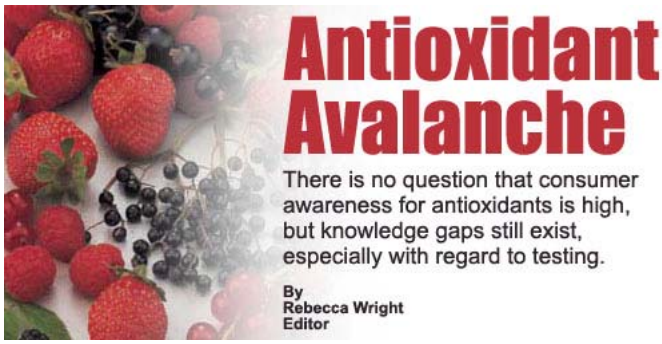
**Where to find:** Other possibilities

Look around:
- vegetables
- fruits
- non-usual edible oils
- spices



Think different
- microalgae
- mushrooms
- berries
- …??

## Other candidates



## OPPORTUNITIES

<u>Critical points</u>

Safety

Evidence

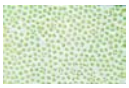
Availability

Regulations

Extraction of compour

Market

New ingredients



## Any case is Different: tocos-sterols-carots-etc

How to start?
Local product (food)
Local source of ingredients (Rosemary case)

Characterize source and components.
Isolate (extract-fractionate) active compounds

Test them. Chemical-invitro-biological activity-
in vivo-clinical: scientific evidence? Articles

Status of clinical studies? Health claims

Source availability-
possibility of industrial production:

What functional food is the ideal for this ingredient?
Available, compatible, complementary
Produce and sell it !

# Research group in functional foods
# Food Science Department
# University Autónoma of Madrid (UAM)

Contact: F. Javier Señoráns
javier.senorans@uam.es

http://**www.uam.es/javier.senorans**

Sec. Dept. de Ciencias de la Alimentación, UAM
Facultad de Ciencias, C-XVI, 502
28049 Madrid (Spain)
http://www.uam.es/otros/gruta/Research/Principal.htm
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