Micronutrients of Citrus fruits : composition, bioavailability and metabolism benefits.

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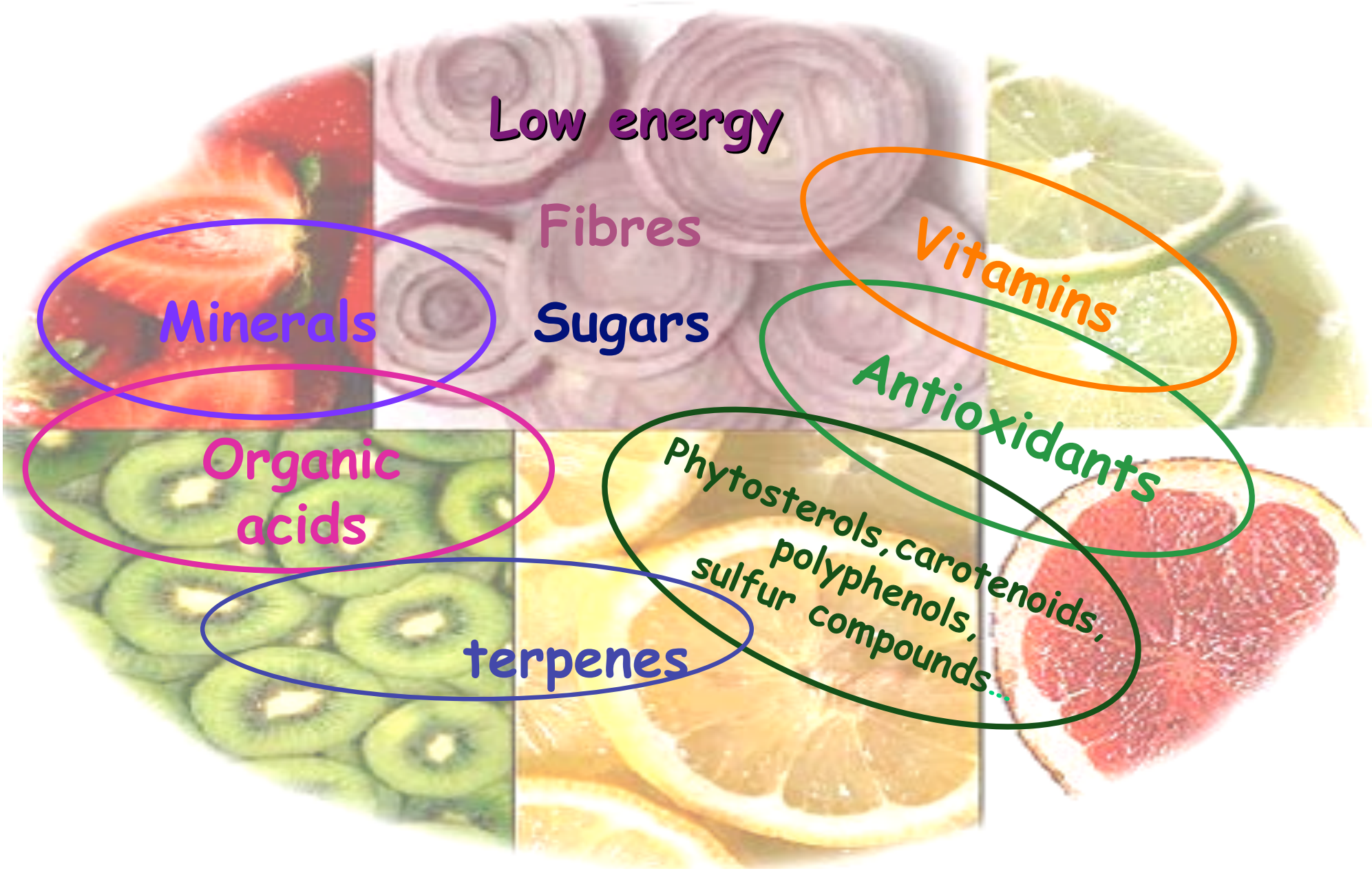
Citrus and Health" meeting, Valencia, 2010 March 8-10

Fruit and vegetables : a complex assembly with low energy and a large diversity of phytochemicals



- Composition in micronutrients and changes
- Bioavailability of micronutrients?
- Bioactivities and metabolic consequences
- Further research

Vitamins, Minerals and Trace elements

Micronutrients : substances that are essential in tiny amounts for the proper growth and metabolism of a living organism. They must be obtained in general from outside sources.

➔Deficiency diseases

➔Increased needs for specific populations

Recommended dietary allowances

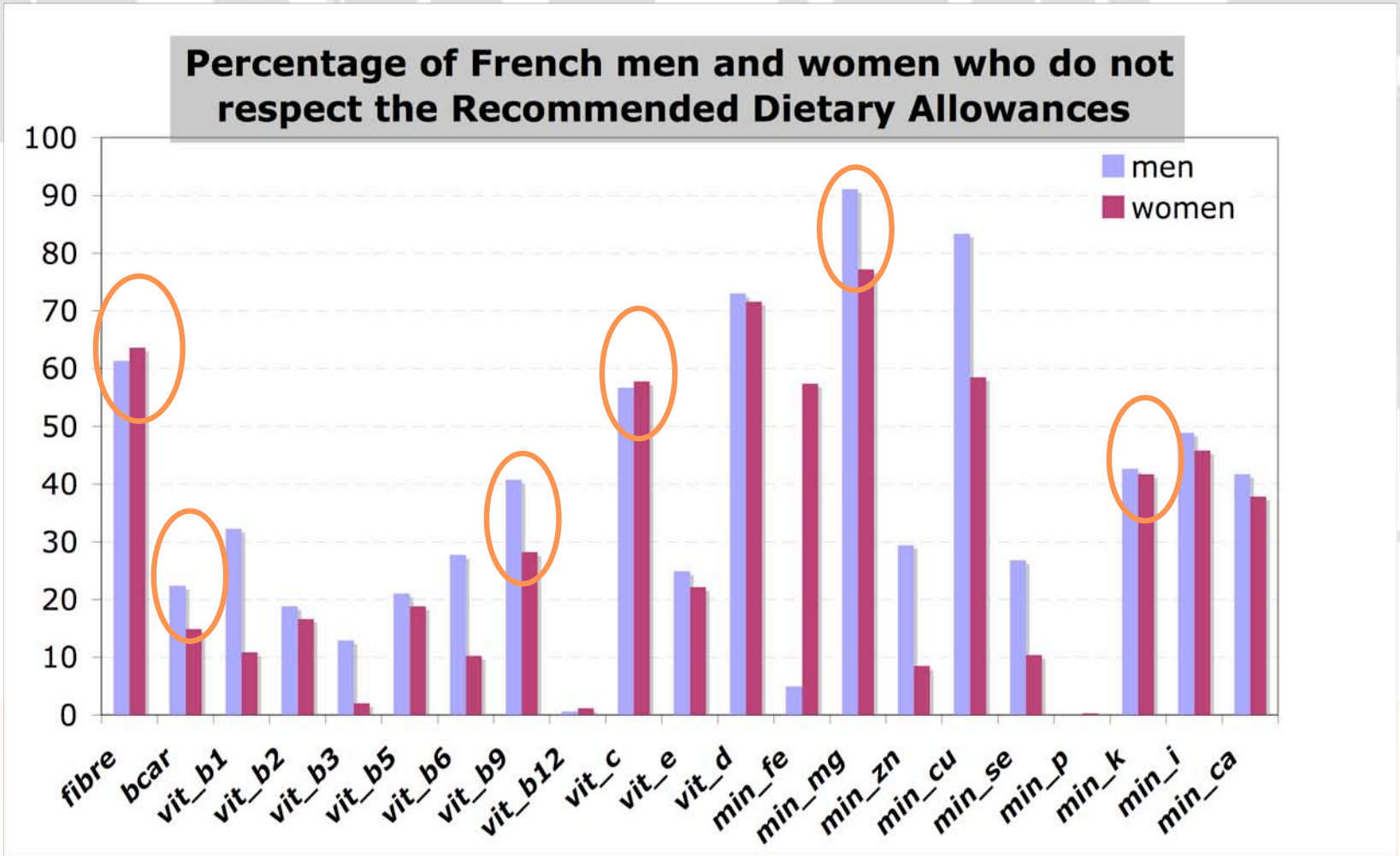
Citrus fruits are rich in Vitamin C, in calcium and in fibers

Components	(g)
Carbohydrates	9
Proteins	1
Lipids	0.2
Water	86
Fibers	**1.8**

Minerals	(mg)
P	16
Ca	**40**
Mg	10

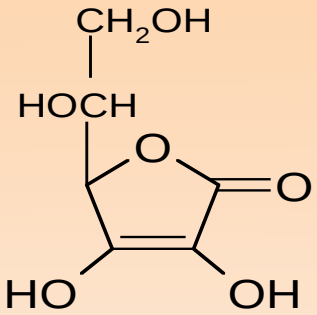
Vitamins	(mg)
Vitamine C (ascorbic acid)	**53**
Provitamin A (carotene)	0.12
Vitamin B1 (thiamin)	0.09
Vitamin B2 (riboflavin)	0.04
Vitamin B3 ou PP (nicotinamide)	0.28
Vitamin B5 (pantothenic acid)	0.30
Vitamin B6 (pyridoxin)	0.06
Vitamin B9 (folic acid)	0.03
Vitamin E (tocopherols)	0.24

Increase of fruit and vegetables intake contributes to reach RDA (fibres and micronutrients)

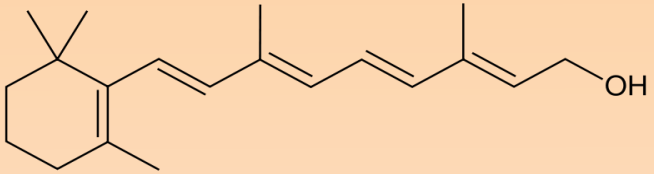


Graphic build on the data food questionnaires of a representative French sub-population of 536 men & 665 women - 18 à 75 year-old (INCA study -Afssa- coordinator: J.L Volatier)

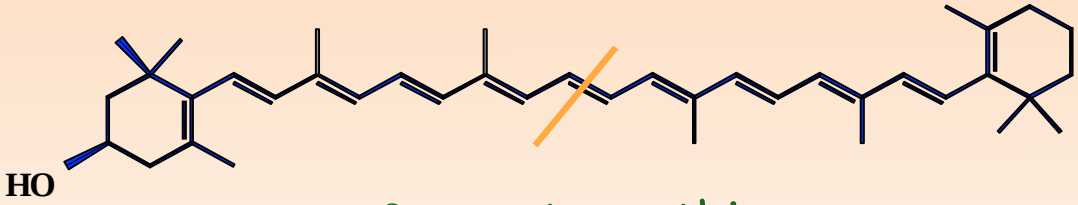
Vitamins A and C



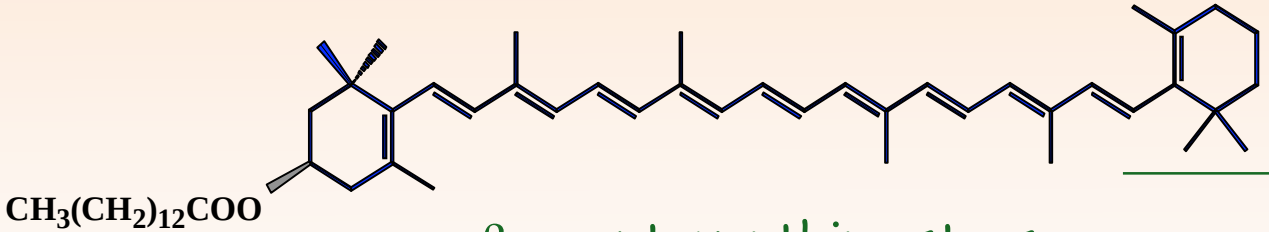
Vitamin C



Retinol
or retinol ester for OH =FFA



β–cryptoxanthin



β–cryptoxanthin esters

	BCX-Esters
Orange (Shamouti)	82-88 %
Mandarin (Hansen)	93-96 %
Lemon (Meyer)	20-25 %

Place of Citrus in food profiling

Nicole Darmon

Some micronutrients are non-essential

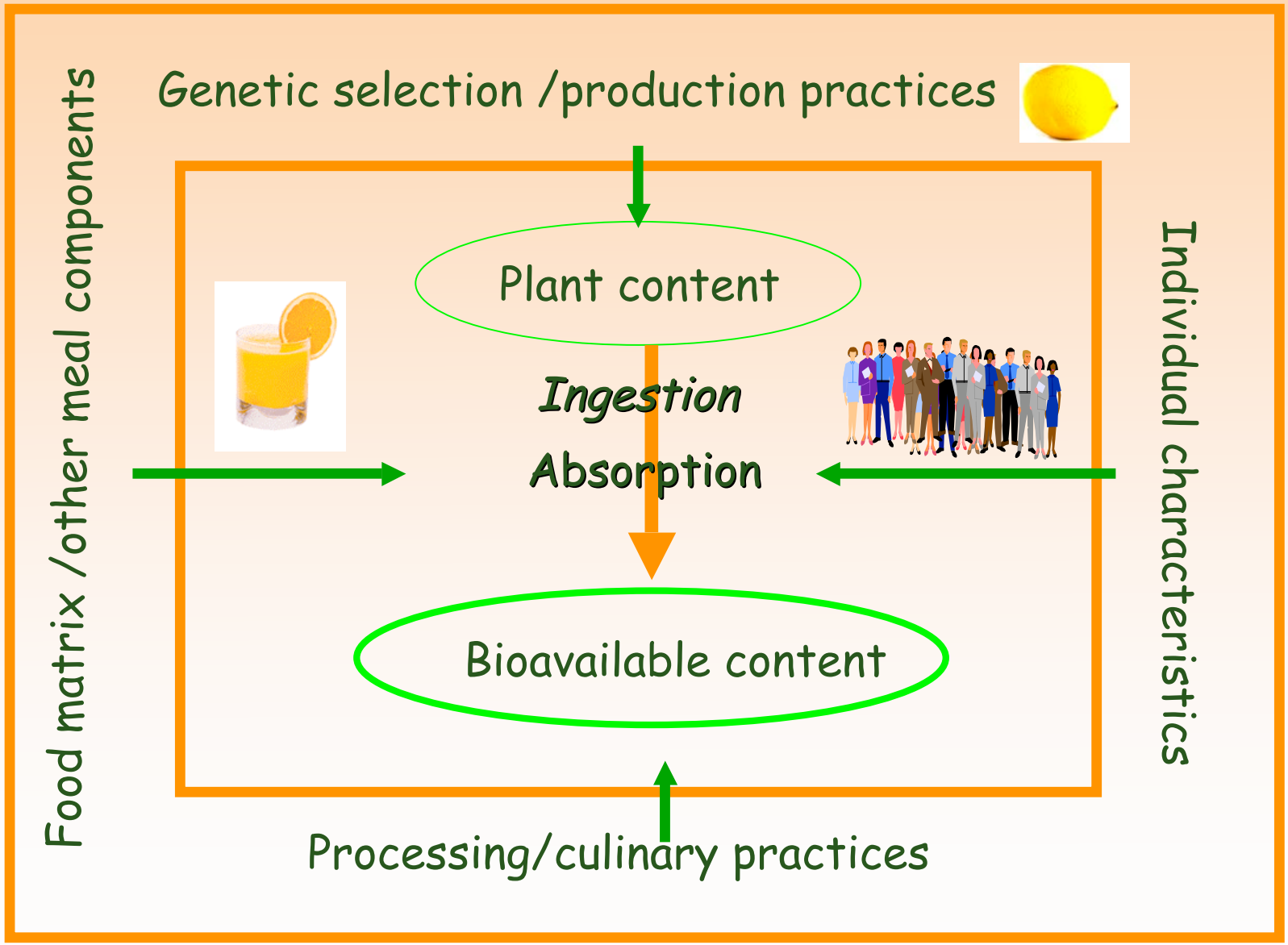
Usually called phytochemicals

Flavonoids, Carotenoids, Terpenoids…

- ➔None deficiency
- ➔Low biological effect
- ➔Chronic age-related conditions

No recommended dietary allowances

Changes in micronutrient level from plant to human?

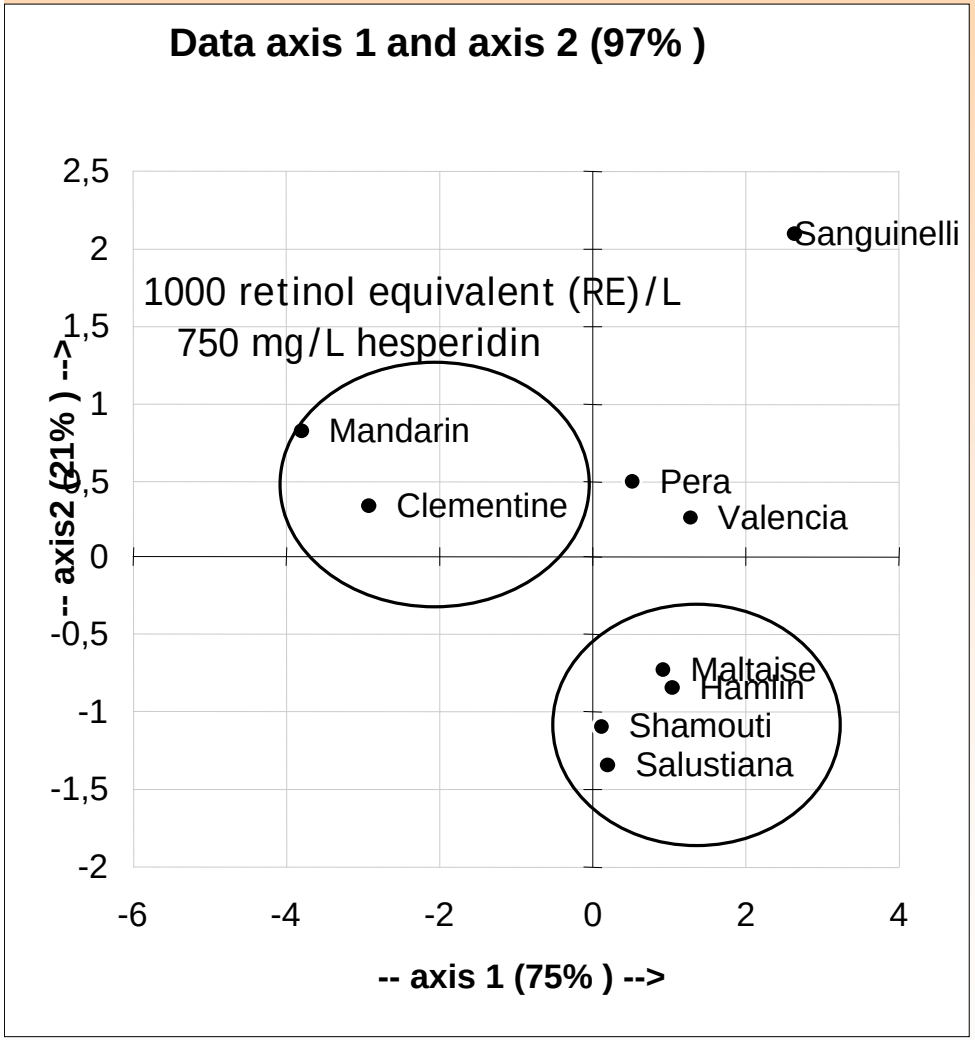


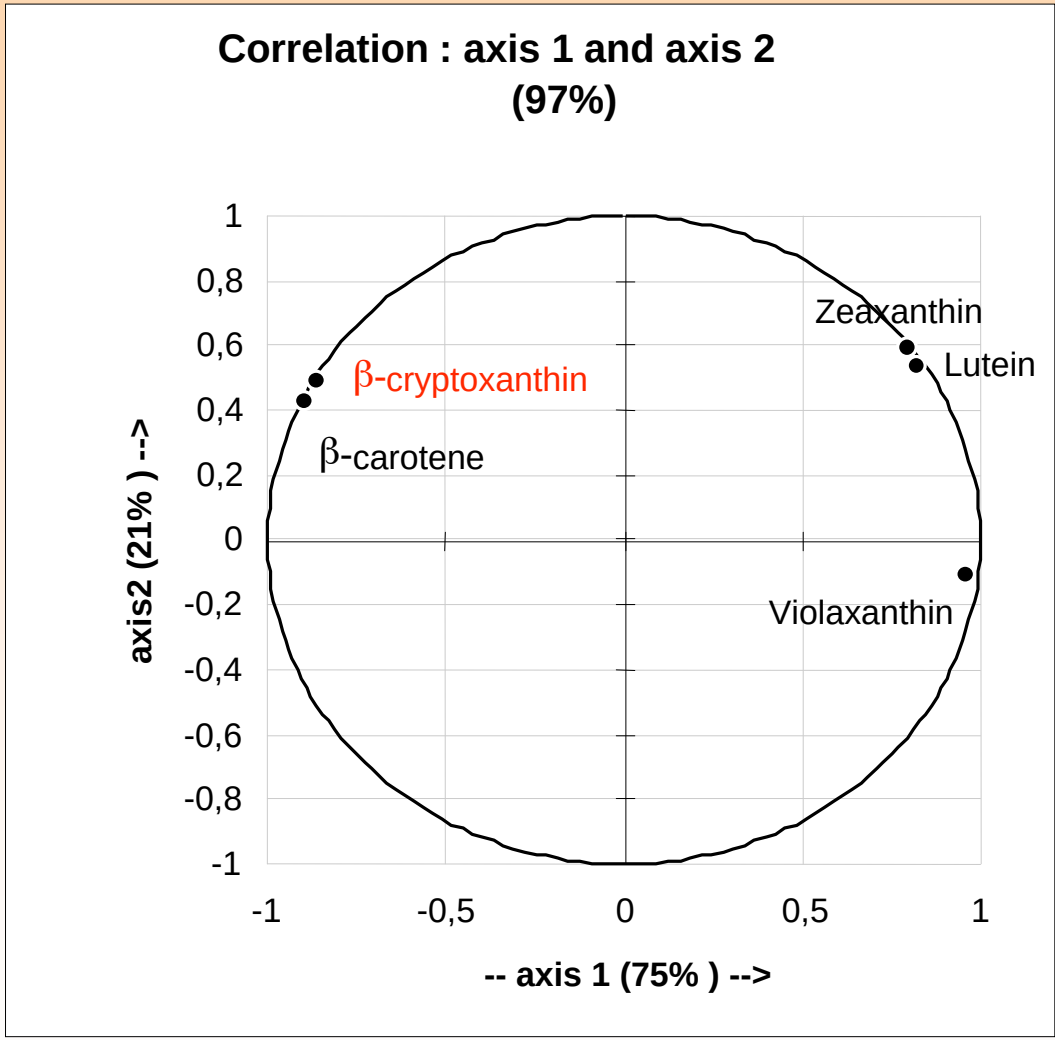
Factors influencing micronutrient contents in plants

	Carotenoids	Vitamin C	Flavonoids
Variety	*** B-cryptoxanthin orange 116-168 mg/100 g Mandarin 560 mg/100 g	*** 40-80 mg/100 g	*** total polyphenols 9-337 mg/100 g Hesperetin 9-41 mg/100 g
Light	*	**	**
Fertilization		** (increase when less N)	** (increase when less N)
Maturity stage	***	*	*

Amiot-Carlin MJ 2003 Quality of plant-derived products. Forum Nutr 56:318-321

Varietal caracterisation of Citrus fruits in relation with antioxidant micronutriment content





C. DHUIQUE-MAYER, C. CARIS-VEYRAT, P. OLLITRAULT, F. CURK & M. J. AMIOT

Journal of Agricultural and Food Chemistry, 2005

Cirad/Inra

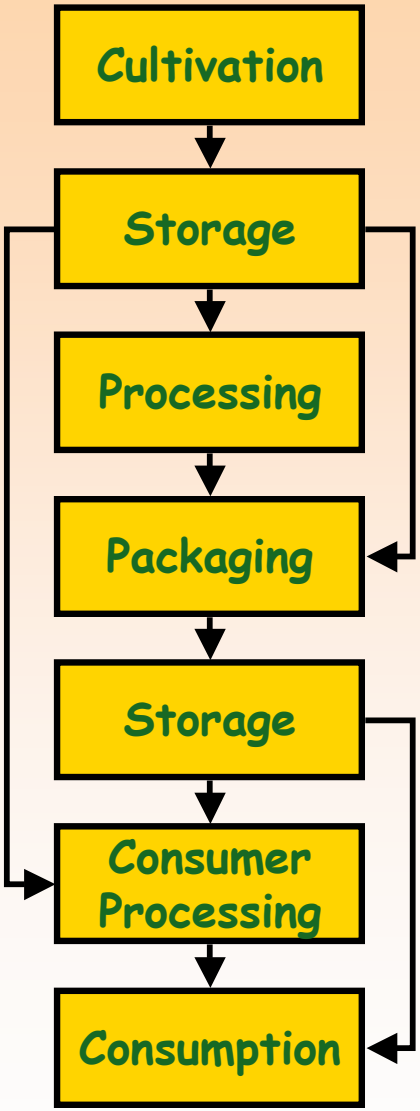
Factors affecting micronutrients during processing

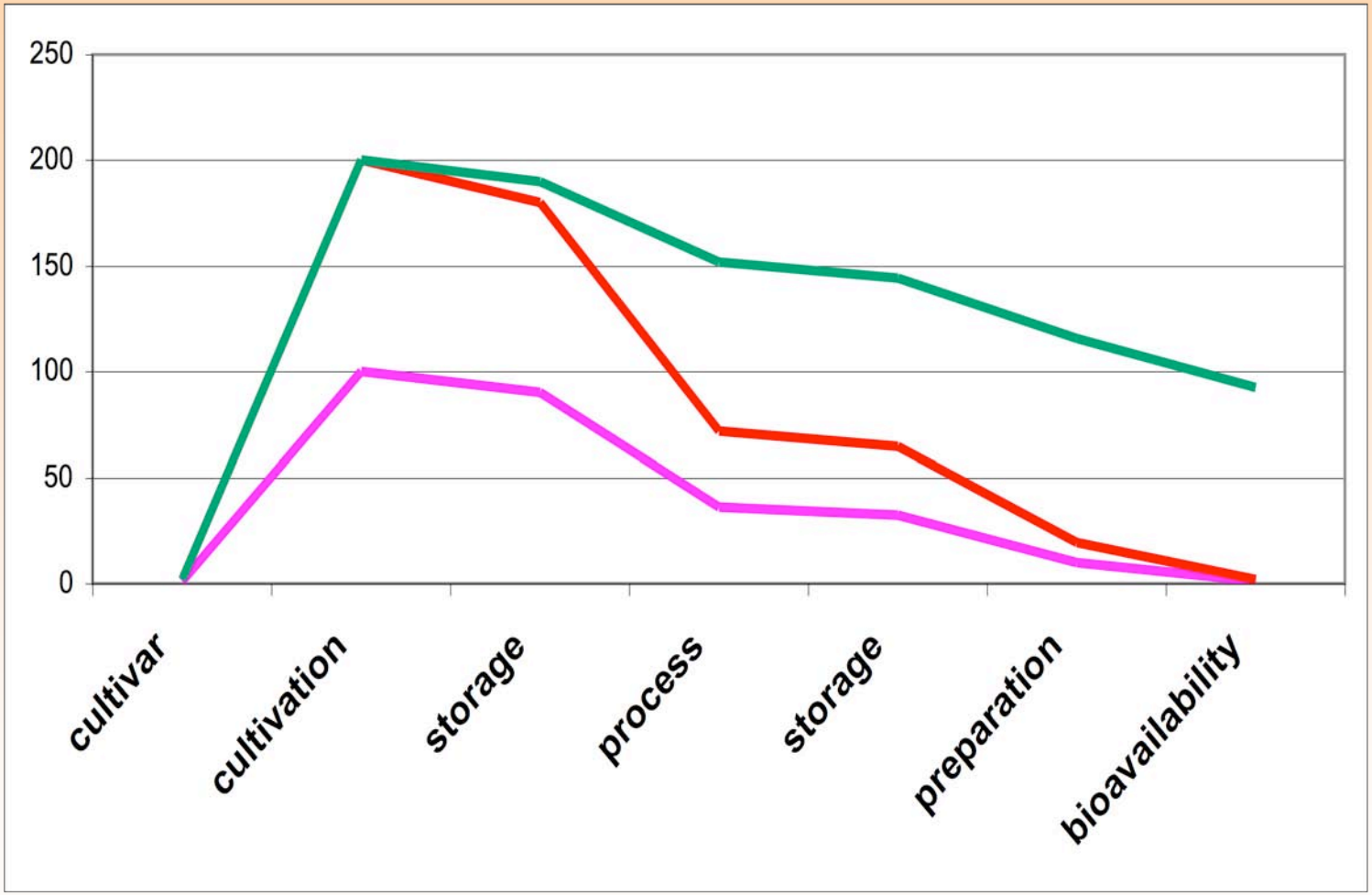
Vitamins	T°	Light	Oxidative agents	Acids	Alkali
Carotenoids					
Flavonoids Except anthocyanins					
Vitamin C					

Very sensitive

sensitive

Hypothetic changes of micronutrients





- Composition in micronutrients and changes
- Bioavailability of micronutrients?
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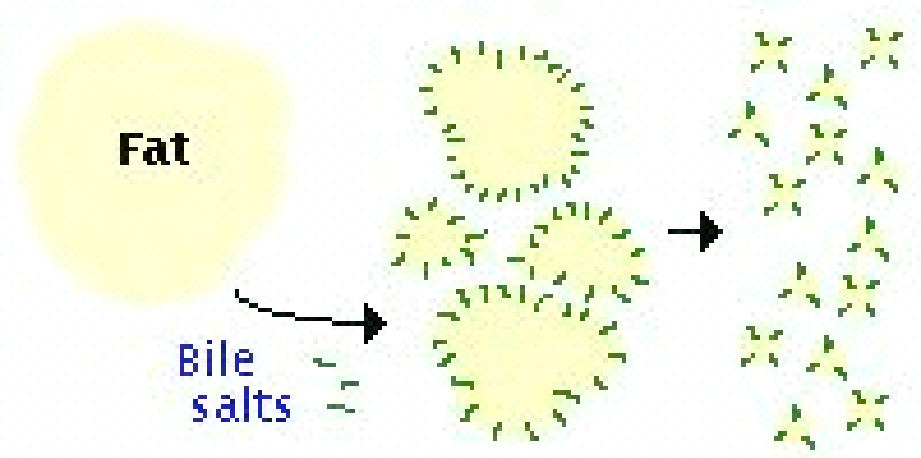
Bioaccessibility

Food matrix

Release of Nutrients and Micronutrients

Proteins /carbohydrates /lipids/carotenoids/vitamins…

Lipophilic Micronutrients (micelles)



Absorption

Intestine

Antioxidant micronutrients along the digestive tract

stomach

Vitamin C >>
Carotenoids
Polyphenols

Scavengers of

RNS and ROS

OH°

RO° RO_2°

H_2O_2

intestine

Absorption

Vitamin C +++
Carotenoids +
Polyphenols +/-

Metabolization
Clivage of
provitaminic A
carotenoids

Conjugaison of
phenolics

colon

Vitamin C low content
Carotenoids and
polyphenols >>

Metabolization
Degradation by
microflora

Scavengers

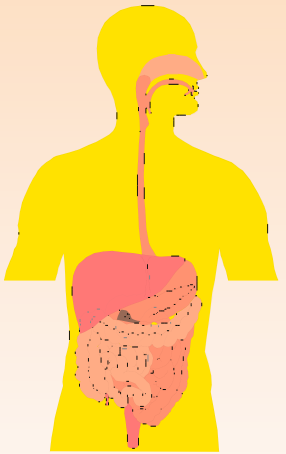
Inhibition of
LOX, COX2

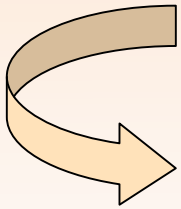
Halliwell et al, 2005, Am J Clin Nutr

What is the behaviour of micronutrients in upper digestive tract?

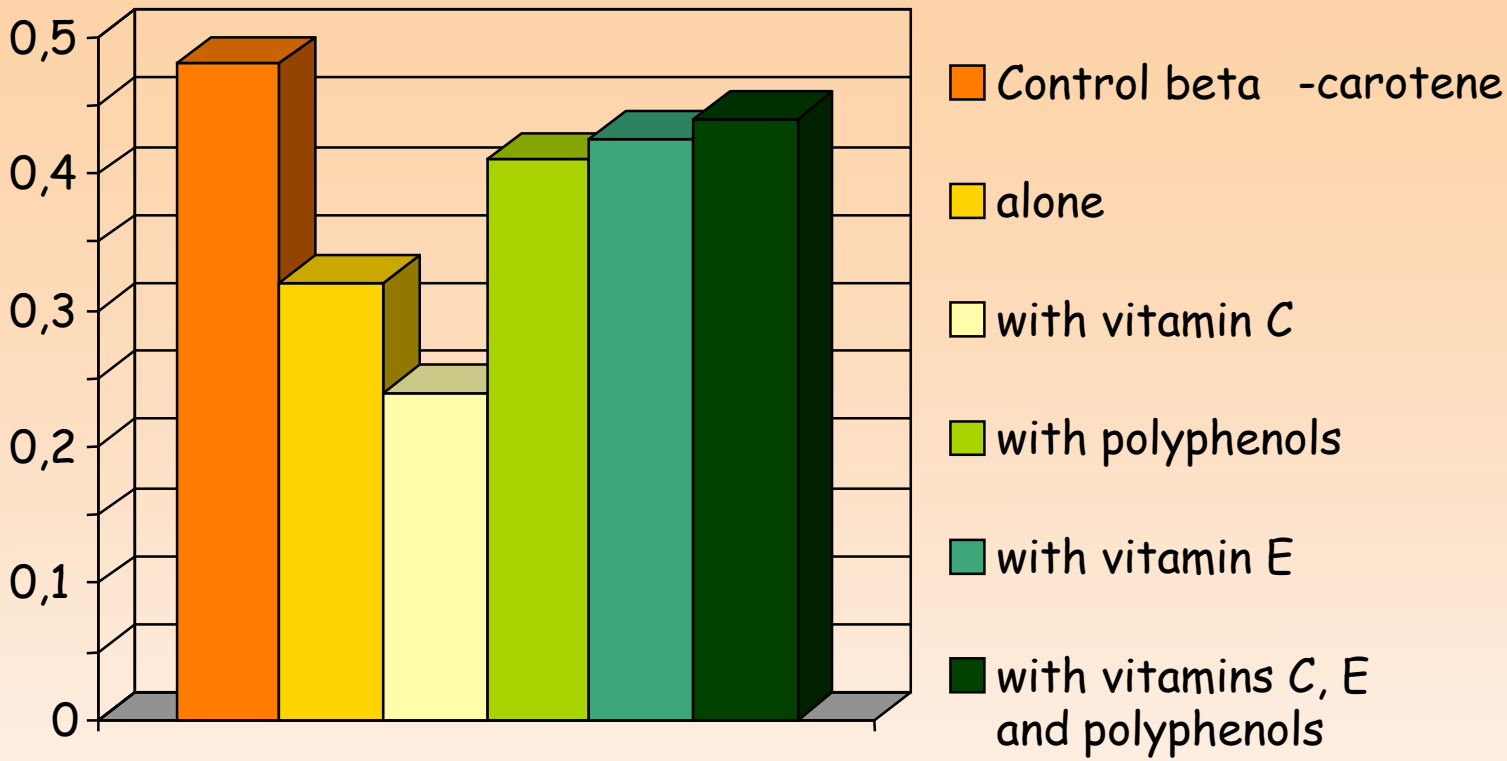
- Design : 16 experiences with
  Vitamin C (100 mg), Vitamin E (12 mg),
  Carotenoids (5 mg) (beta carotene, lycopene and lutein)
  Polyphenols (1g) (gallic acid, caffeic acid, flavanone and flavane)

- Physiological conditions
- Daily intake/micronutrient





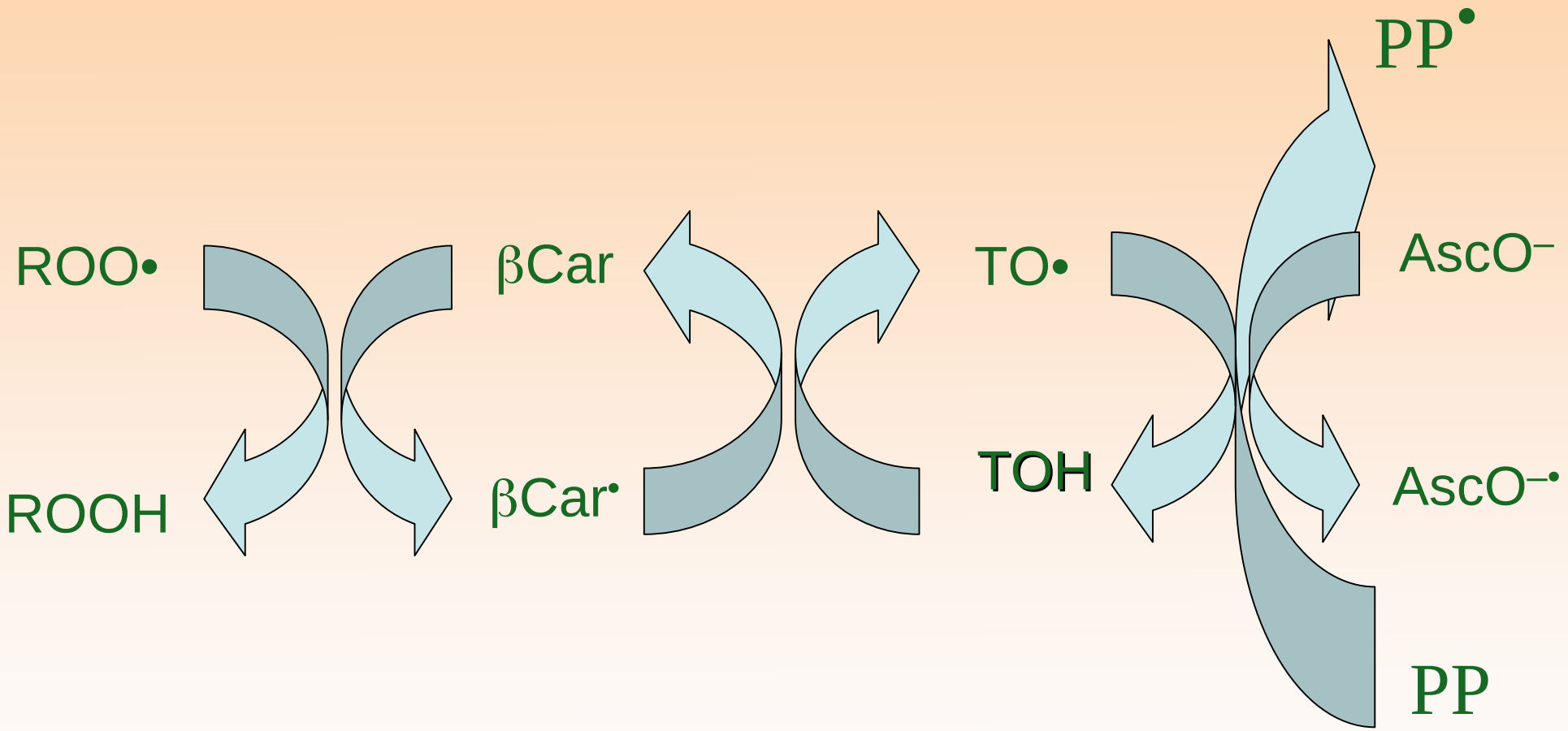
Our recent works support a protective and synergistic effect of the main antioxidants present in our dietat the pre-absorptive level



Results on carotenoids : beta-carotene is less stable with vitamin C and more stable in stomach and duodenum when combined with phenolic compounds

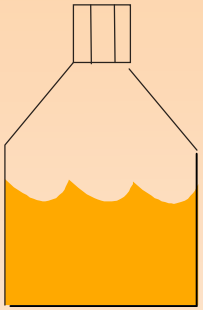
In general, stability of micronutrients is greatly improved when combined

Inter-dependence of oxidative reactions



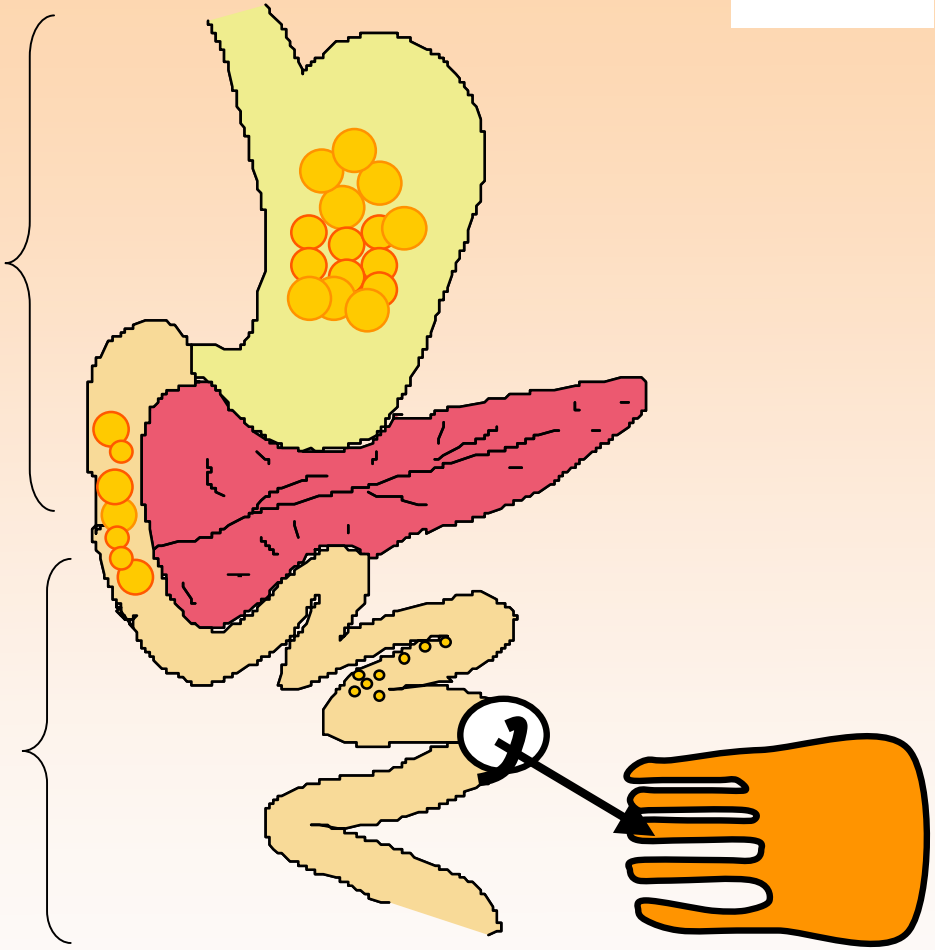
Synergistic effects of diverse micronutrients present in fruits and vegetables

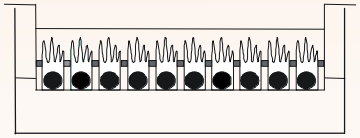
Two steps and two models to assess bioavailability



(bioaccessibility)

Acellular model: *In vitro* digestion



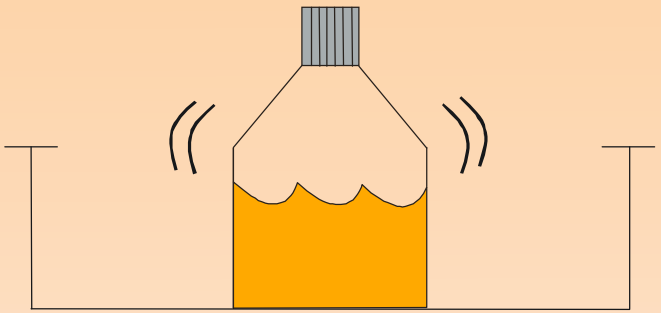


(intestinal uptake)

Cellular model: Caco-2 (TC7 clone)

Enterocyte

In vitro digestion/caco-2 cell culture model



Gastric step digestion

Ph 4, pepsin, 37°C, 30 min

↓

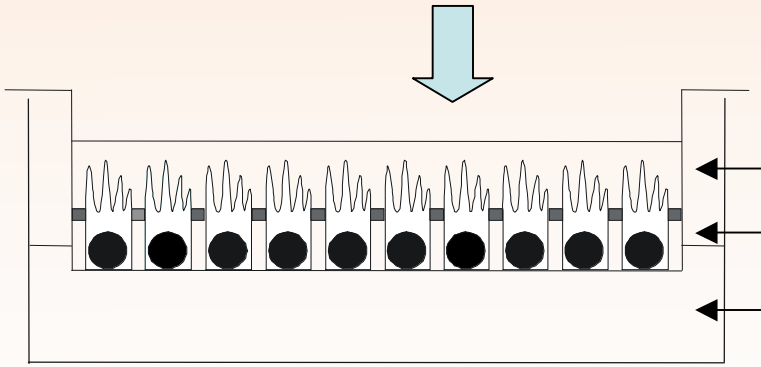
Intestinal step digestion

Ph 6, Pancreatin-bile extract, 37°C, 30 min

↓

Centrifugation-filtration (0.2µM)-dilution

Carotenoid-rich Micelles (2h30-incubation)



Apical medium

Differentiated Caco-2 cell monolayer

Basolateral medium

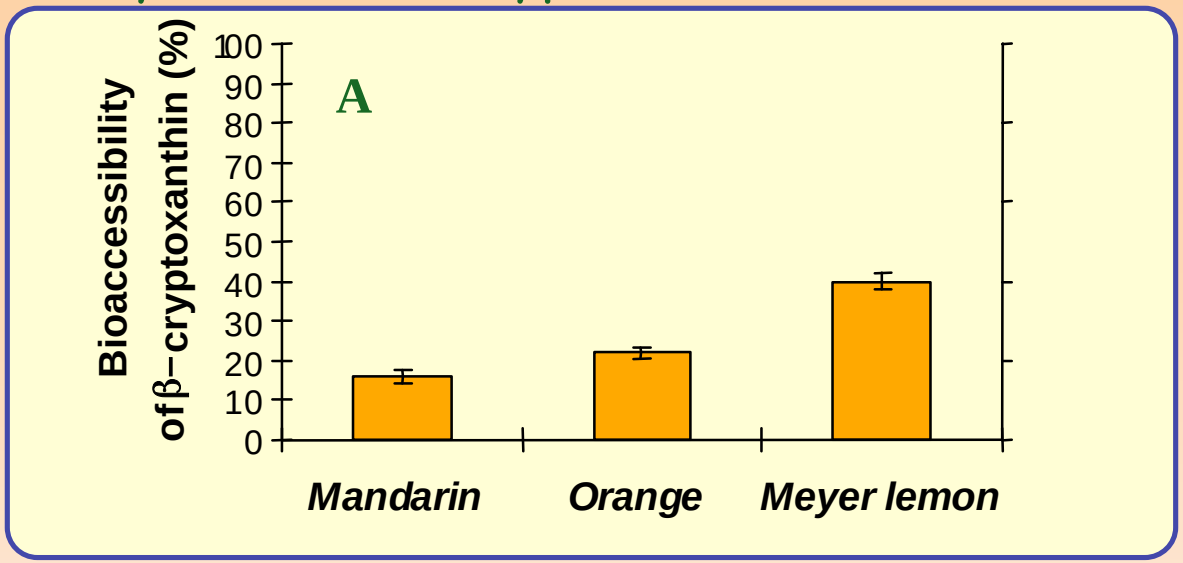
Free and esterified b-cryptoxanthin in native and digestive juices

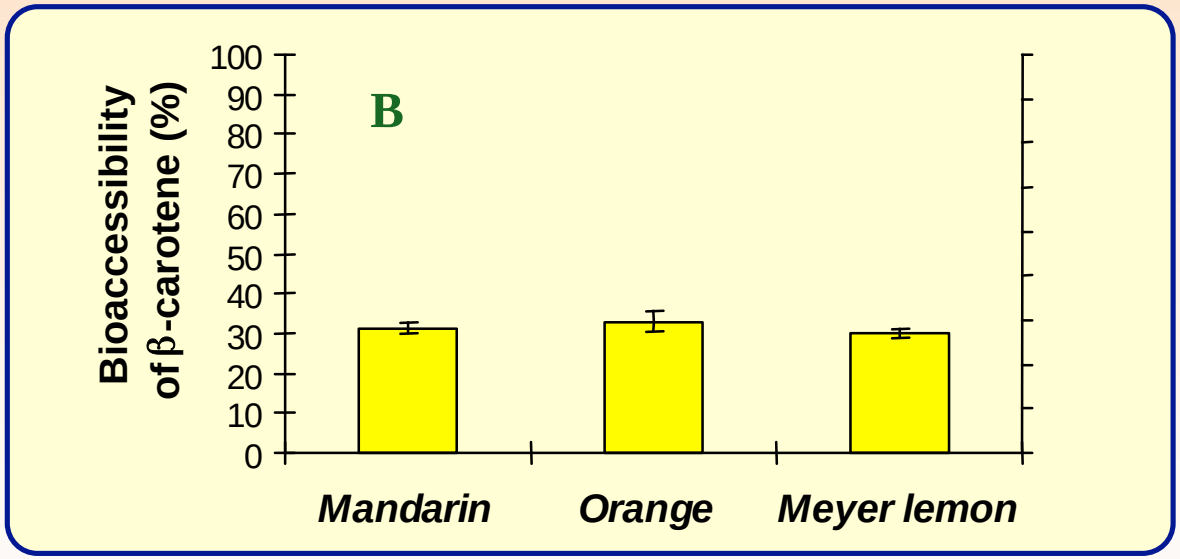
	ß-cryptoxanthin in native juice (mg/200ml)‡			ß-cryptoxanthin in digested juice (mg/200ml)‡		
	Free Bcx	BcxL	BcxM	Free Bcx	BcxL	BcxM
Orange	0.16ƒ 0.02 (18 %)	0.25 ƒ 0.03 (29 %)	0.47 ƒ 0.13 (53 %)	0.23 ƒ 0.02 (62 %)*	0.04 ƒ 0.004 (11 %)	0.1 ƒ 0.008 (27 %)
Mandarin	0.17 ƒ 0.06 (5 %)	1.28 ƒ 0.28 (39 %)	1.83 ƒ 0.32 (56 %)	0.32 ƒ 0.03 (20 %)*	0.50 ƒ 16 (31 %)	0.78 ƒ 0.28 (49 %)
Meyer lemon	0.18ƒ 0.03 (75 %)	0.03 ƒ 0.008 (11 %)	0.03 ƒ 0.006 (14 %)	0.19 ƒ 0.06 (86 %)*	0.01 ƒ .002 (5 %)	0.02 ƒ 0.003 (9 %)

· Mean of three determinations mg/200ml± SD; *significant difference ($p < 0.05$)

Digestion liberates free form of beta-cryptoxanthin

Bioaccessibility of total b-cryptoxanthin (A) and b-carotene (B)





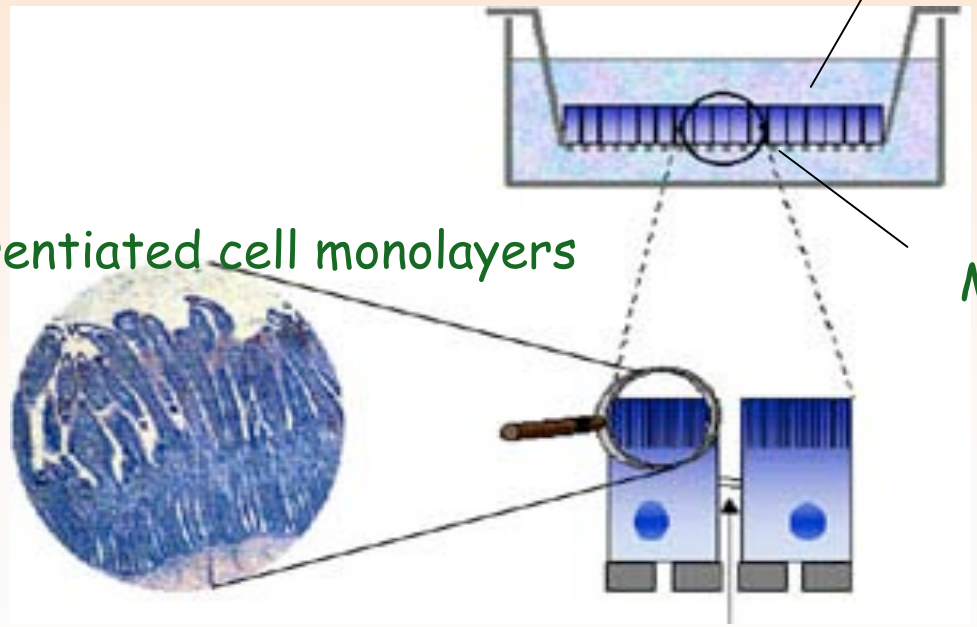
Micellarization is an important factor for the bioavailability of carotenoids

Beta-cryptoxanthin is better transferred into micelles than beta-carotene , than esters of beta-cryptoxanthin

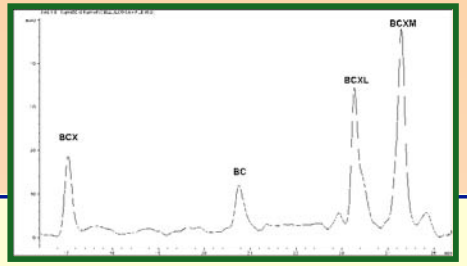
Caco-2 (clone TC7) cell culture

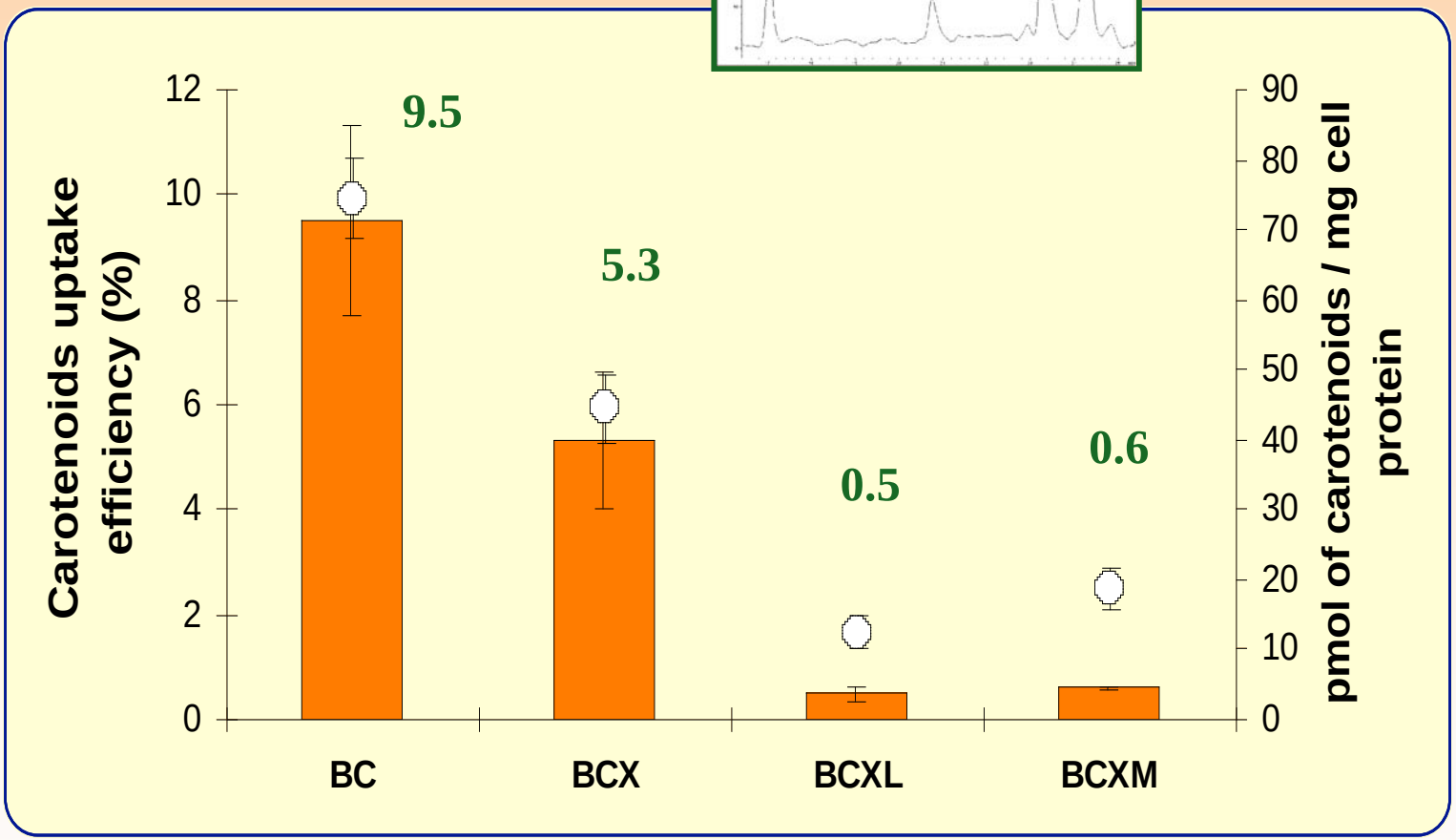
Confluence after 7 days

21 days on transwells



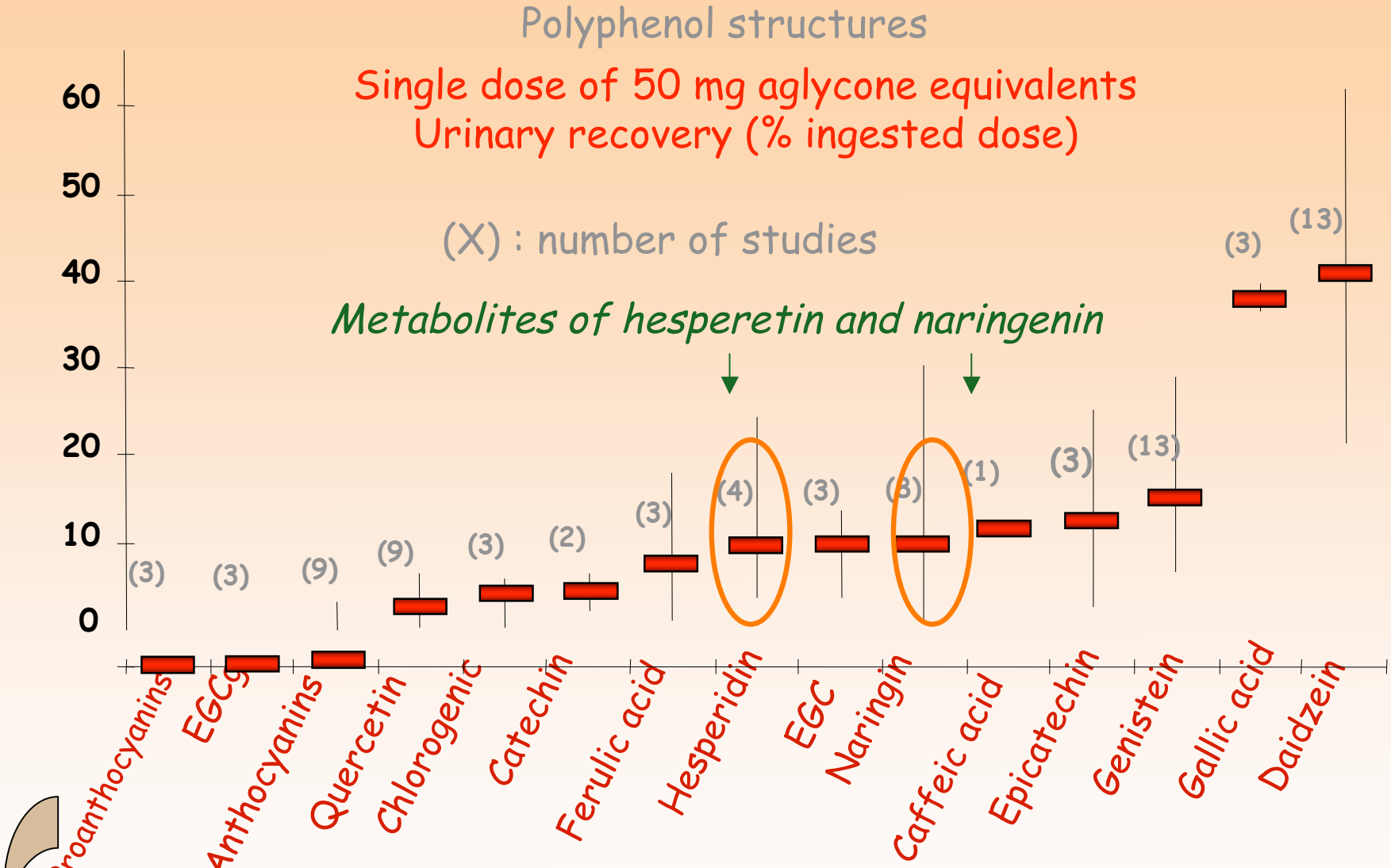
Uptake of carotenoids by Caco-2 cells





Beta-carotene is more absorbed than beta-cryptoxanthin, which is, as free form, more absorbed than the esters

Hesperidin and naringenin are poorly absorbed as the majority of phenolic compounds and highly metabolized



(Manach et al., 2005, Human Nutrition Unit in Clermont_Ferrand, France)

Bioactivity of Citrus flavonones is different than direct antioxidant mechanisms after absorption

- Composition in micronutrients and changes
- Bioavailability of micronutrients?
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- Further research

Plasmatic concentrations of antioxidant micronutrients

Antioxidants	Concentrations micromol/L	Coefficient of variation %
Vitamin C	60	82
α-tocopherol	17	33
Flavonoids	0,001 à 4 (according to structures) Hesperetin (1)	73 à 231
Carotenoids	2 β-cryptoxanthin (0,27)	426 (F)

+ Endogenous antioxidants (uric acid, glutathione, albumin and bilirubin)

Micronutrients, especially non-essential micronutrients, probably act as signalling molecules

Metabolic consequences

Intake	Sujects	Endpoint	Authors
+ Citrus juice But not vitamin C alone	with hypercholesteromia	Diminution of TG	Vinson & Jang, 2001
+ Mandarin fruit	non-smoking female	Increase of beta-cryptoxanthin Associated to HLD-cholesterol	Sugiura et al, 2004
+ Grapefruit	with atheroslerosis	Diminution of LDL-cholesterol and TG	Gorinstein et al, 2006
+ Grapefruit	Obeses	Weignt loss Improvement of Insulin sensitivity	Fujioka et al, 2008

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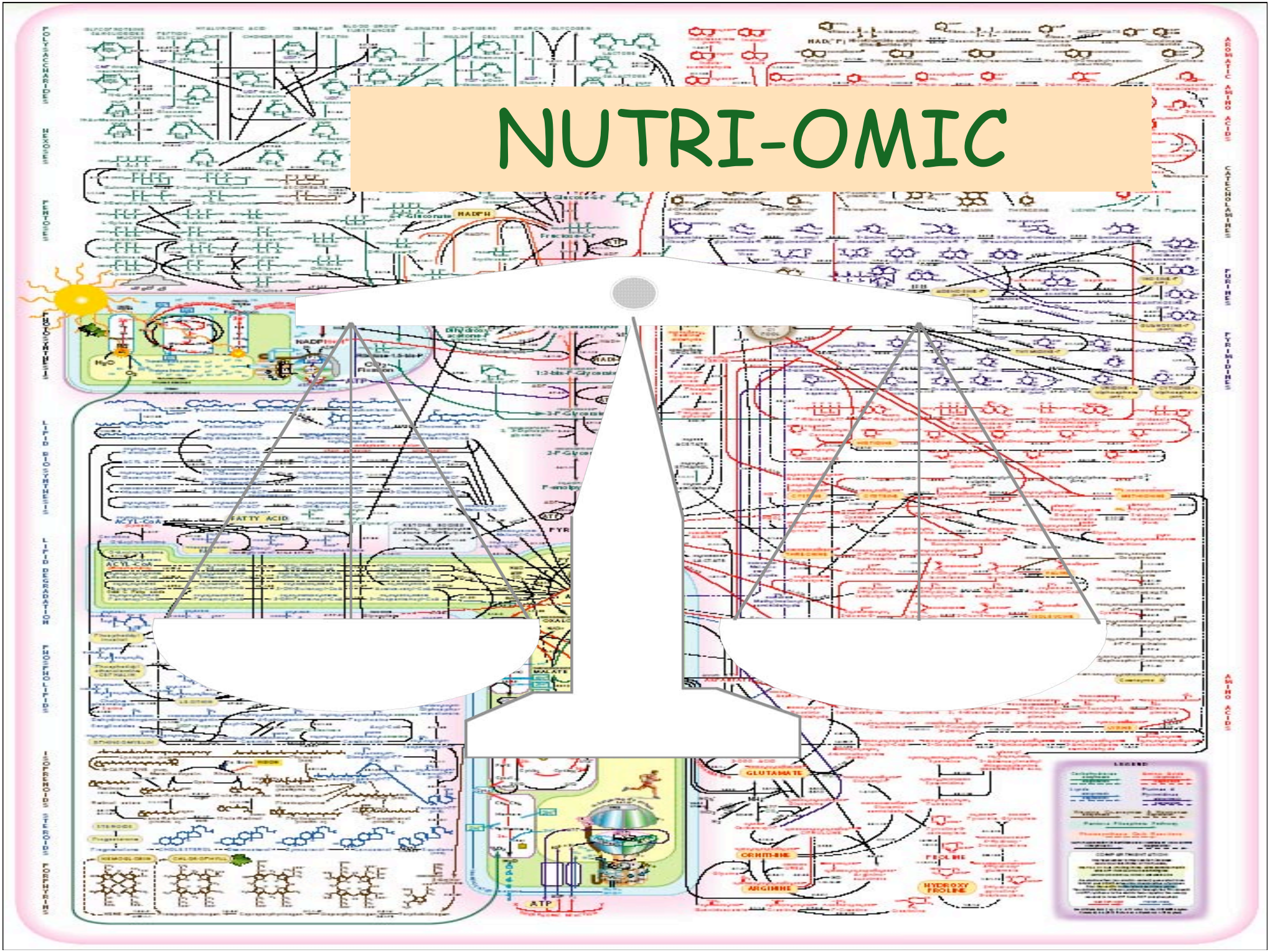
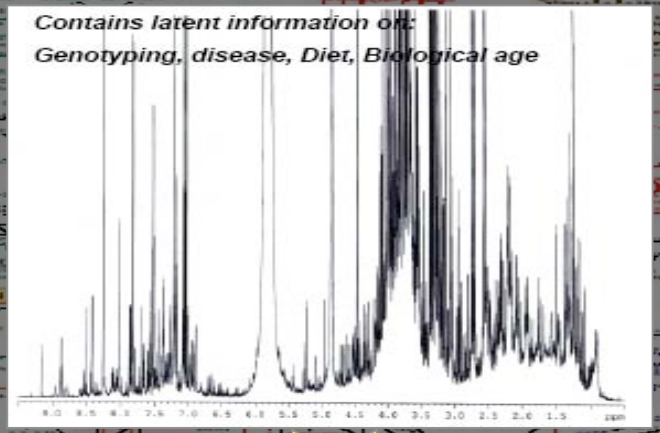
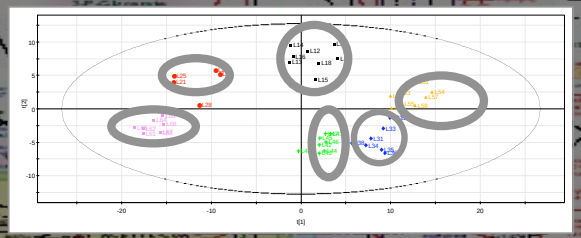

NUTRI-OMIC
POLYSACCHARIDES
HEXOSES
PENTOSES
PHOTOSYNTHESIS
LIPID BIOSYNTHESIS
LIPID DEGRADATION
PHOSPHOLIPIDS
ISOPRENOIDS
STEROIDS
PORPHYRINS
AROMATIC AMINO ACIDS
CATECHOLAMINES
PURINES
PYRIMIDINES
AMINO ACIDS
NADPH
ATP
FATTY ACID
ACYL-CoA
GLUTAMATE
ARGININE
HYDROXY PROLINE

RMN
LC/MS
GC/MS

plasma, urines, tissues



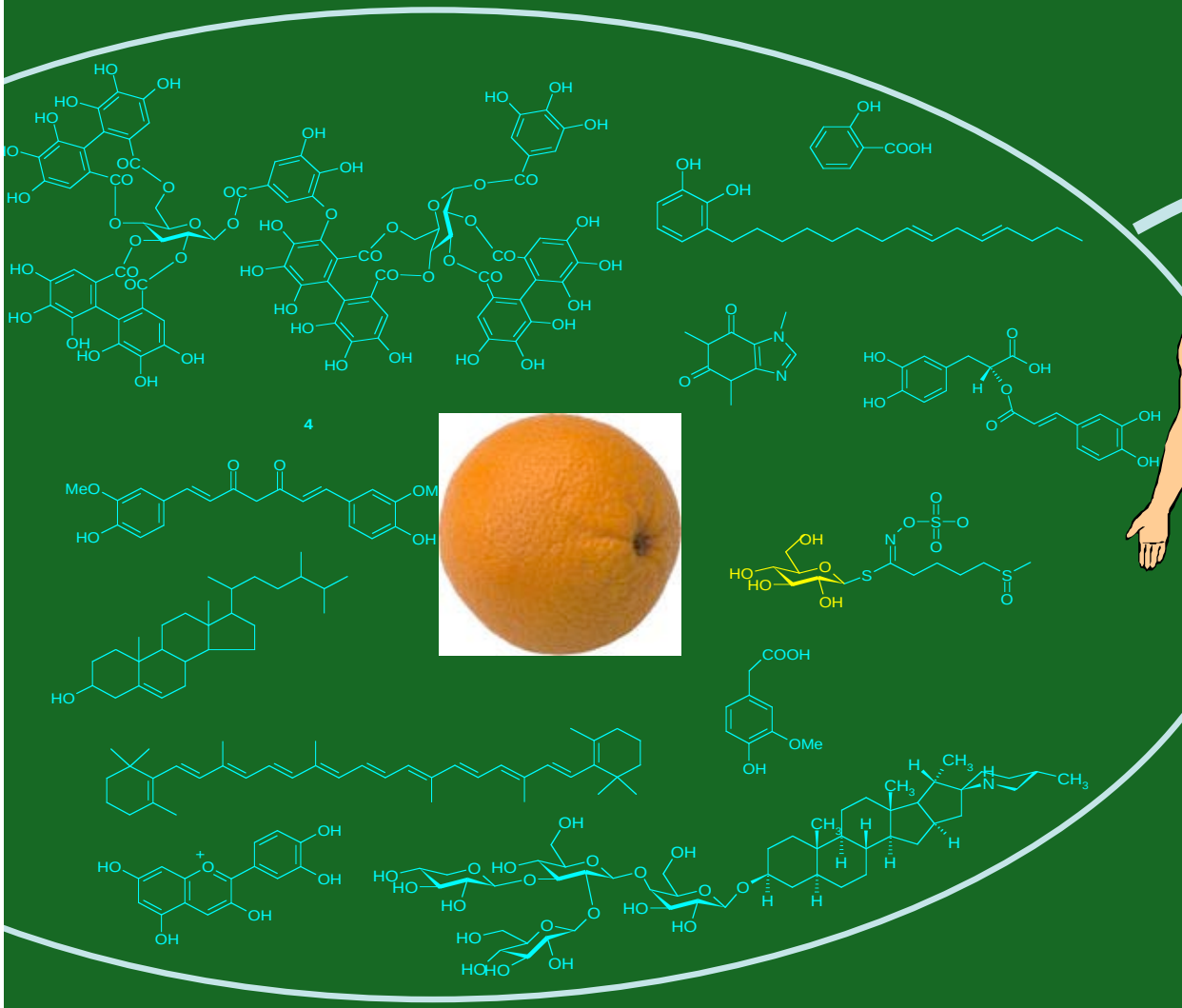
Data acquisition



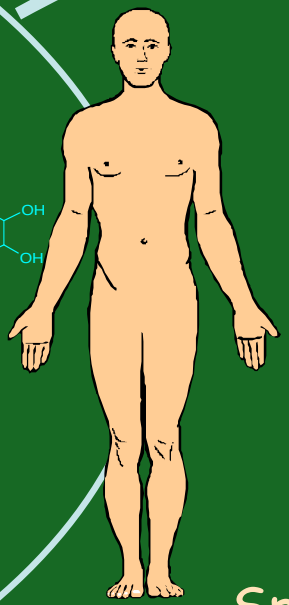
Statistics

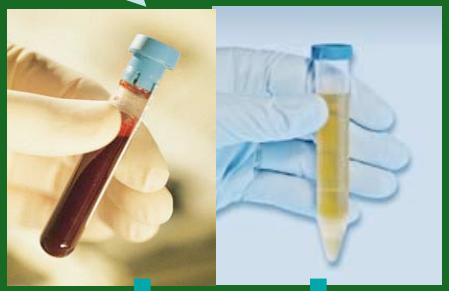
« Data mining »:
Metabolites
Metabolic pathway

Food Metabolome



Absorption
Metabolism
Excretion

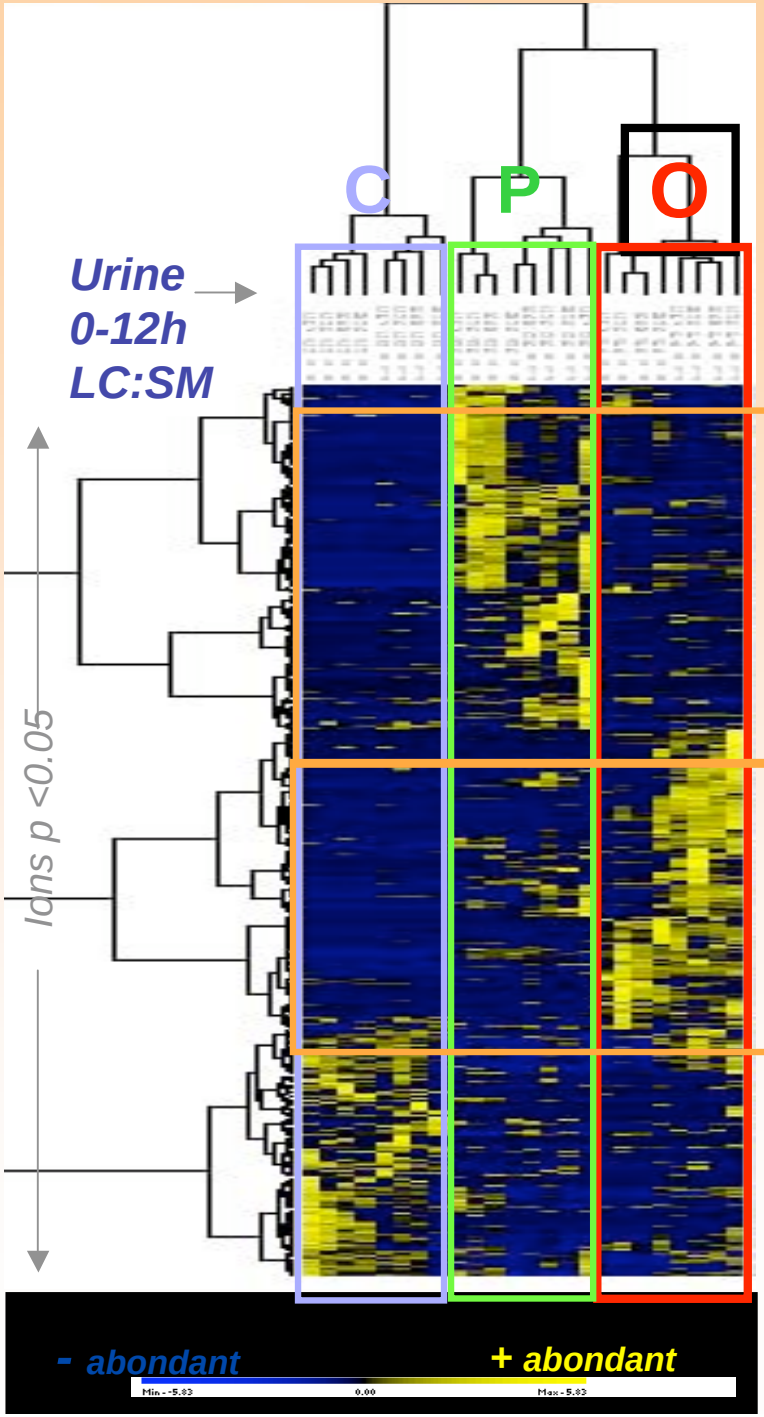




Specific signatures
of food consumption

Manach et al., 2009, *Mol Nutr. Food Res.*

Biomarkers of consumption



Cluster analysis

477 ions after grapefruit juice consumption

+ 214 ions common

358 ions after orange juice consumption

Biomarkers of effects?

Global approach

Claudine Manach, INRA UNH, Clermont

New identified biomarkers

Orange

Hesperetin glucuronides
Hesperetin sulfate
Naringenine glucuronide
Stachydrine
Aucubin
Limonene epoxide
Dihydroperillic acid
Tetramethoxyflavone
Dihydroxypentamethoxyflavone
Dimethoxycoumarin glucuronide
Methyltyramine sulfate
Confertifolin
Myrcene diol
…

Grapefruit

Naringenin glucuronides
Eriodictyol glucuronide
Bergaptol glucuronide
Stachydrine
Aucubin
Scopoletin
Demethylnobiletin
Dihydroxypentamethoxyflavone
Trimethoxycoumarin glucuronide
Myrcene Hydroxy Acid
Limonene epoxide
Nootkatone
Hydroxycarvone
…

WHAT HEALTH!

Thank for attention