

**Main class of anti-oxidant**  
(Polyphenols and carotenoids) of Indigenous fruits


Stress antioxidant

Polyphenols chemistry and « polyphenols classes » & Aspects of analysis and main problems

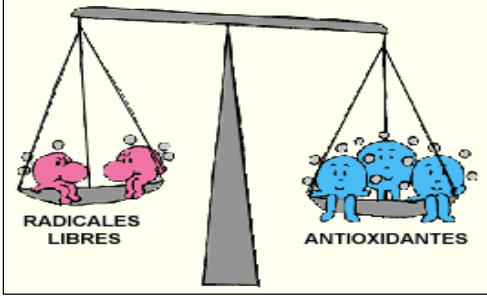
Carotenoids chemistry and « carotenoids classes » & Aspects of analysis and main problems

ORAC interest and Bioavailability...

documents Autors presented:  
E.Rock, Guyot, (Inra Clermont, France),  
Kimura, M. & Rodriguez-Amaya, D.B. (1999), Brazil;  
C.Mertz, F.Vaillant, P.Bratt, cirad, France,  
F.Hau, University Barquisimeto Venezuela.



Pictures/ E.Rock, Inra Clermont



**antioxidants against oxidative stress**  
(ref/E.Rock/Inra Clermont/France)

UN ATOMO NORMAL

LA PERDIDA DEL ELECTRON CONVIERTE ATOMO SANO EN RADICAL

Energy

Oxygen singlet  $O^{\bullet}$

Hydroxyl radical  $OH^{\bullet}$

Hydrogen peroxide  $HOOH$

peroxynitrite  $NOO^{\bullet}$

Reactive Oxygen Species

ROS

Mammals including human beings are adapted to live in oxygen containing atmosphere.

Oxygen molecule is somewhat unstable and is at the origin of reactive oxygen species

Atmospheric oxygen can be easily transformed into reactive species

Formes allotropiques de l'oxygène

2p	↑↓	↑↓	↑↓	↑↓	Anion Superoxyde
2s	↑↓	↑↓	↑↓	↑↓	
1s	↑↓	↑↓	↑↓	↑↓	
2p	↑↓	↑↓	↑↓	↑↓	Oxygène Singulier
2s	↑↓	↑↓	↑↓	↑↓	
1s	↑↓	↑↓	↑↓	↑↓	
2p	↑↓	↑↓	↑↓	↑↓	Oxygène triplet
2s	↑↓	↑↓	↑↓	↑↓	
1s	↑↓	↑↓	↑↓	↑↓	

**Main antioxidants in fruits**

**The Color Guide**

Ref: E.Rock-INRA France 2009

Green	Yellow Orange	Red	Blue Purple	
Lutein Zeaxanthin Indoles Vitamin K Potassium	Beta-Carotene Vitamin A Bioflavonoids Vitamin C Potassium	Vitamin C Lycopene Anthocyanins	Anthocyanins Vitamin C Phenolics	Allium Allicin
Spinach Cabbage Lettuce Broccoli	Carrots Oranges Banana	Radishes Tomatoes Strawberries	Blueberries Eggplant	Garlic Onions

The trends are on the study focused on phytochemicals (i.e. polyphenols & carotenoids) rather than minerals and vitamins

**Oxidative stress: biological concept**  
(ref/E.Rock/Inra Clermont/France)

ROS

ANTIOXIDANTS

Respiratory chain (mitochondria)  
Immunity (leukocytes)  
Detoxification (microsomes)  
Pollution, irradiation

Enzymes (SOD, Cat., GPx, GR, ...)  
Proteins (albumin, caeruloplasmin, ferritin, ...)  
Nutrients and non protein compounds  
**vitamins, carotenoids, minerals, flavonoids**  
Uric acid, GSH, ...)

Imbalance

**Oxidative stress**

Oxidative stress is an unbalanced situation

**Polyphenols????** ( ref/ Guyot/inra France)

Polyphénols  
Phenolics compounds  
Tannins  
Tannoïdes

?

Polyphénols = Phenolic compounds

Tannins : particular class of polyphenols, do association with proteins

**POLYPHENOLS IN THE FOODS**  
**main combinations**  
 (ref/E.Rock:Inra Clermont/France)

**Esterification**

**Glycosylation**

**Polymerisation**

**Some chemistry (2)** (ref: Guyot and al /inra-France)

**structure and functionality**

**Hydrophil groups**

**Hydrophob group**

- Physico-chimistry : **solubility**, interact with polysaccharides, protéins...
- Biochimistry reactivity:oxydation, etc...

**Main class of polyphenols**

**Main class of polyphenols:**

**simple polyphénol and phenolic acid**

flavonoids

coumarins & naphthaquinones

tanins

**Main class of polyphenols of interest in fruits**  
 (ref Wikipedia/ internet)

**Phenols acid - simple**

**Phenolic acids**  
 C6-C1ou C6-C3

Caffeic acid      Benzoic acid      Gallic acid      Vanillic acid

**Some chemistry (1)** (ref: Guyot and al /inra-France)

**Phenol group**

**Basic structure of this phenolic compound**

R : can be a sugar, organic acid

**Ex : Catéchin**

**POLY phénols**

**Main class of polyphenols**

**Main class of polyphenols:**

simple polyphénol and phenolic acid

**flavonoids**

coumarins & nathataquinones

tanins

**Main class of polyphenols of interest in fruits**  
(ref Wikipedia/ internet)

**Flavonoids(anti-oxydants+++)** with or no aglycone, heterosides, methoxylation)

**Flavonoids**  
C6-C3-C6

Flavonols  
Flavones  
Isoflavones  
Flavanones  
Flavanols  
Anthocyanids

Flavanone /heterosides form::  
hesperidine, naringine)

Flavonol ( rutine)  
Aglycones: quercetol, kameferol

Quercetol or (Quercetine)  
(aglycone form)

**Main class of polyphenols of interest in fruits**  
(ref Wikipedia/ internet)

**Coumarins and naphtha quinones**

Coumarine aromatic compounds

Naphta quinone  
cytotoxic, anti bacterian,,anti fungi,

**Main class of polyphenols of interest in fruits**  
(ref Wikipedia/ internet)

**Flavonoids(anti-oxydants+++)**

Flavanols , anthocyanidols (with or no aglicones, gallates, polymeres, heterosides)

Catéchine  
heterosid form: epicatechin

Anthocyanidol : aglycone form:  
exemple: pelargonidol, delphinidol

or  
Anthocyanidin: hererosides forms:  
exemple : anthocyanes cyanidol 3,5 di glucoside

**Main class of polyphenols**

**Main class of polyphenols:**

simple polyphénol and phenolic acid

flavonoids

**coumarins & nathataquinones**

**tanins**

**Main class of polyphenols of interest in fruits**  
(ref Wikipedia/ internet)

Flavonol

Flavone

Flavanone

Dihydroflavonol

Anthocyanidines

Flavan-3-ol

Flavan-3,4-diol

**Main class of polyphenols of interest in fruits**

**Tanins... class**

Acide ellagique

Gallo tanin

**Tanins complex:**  
- ellagitanin and epicatechine  
- condensed tanins : flavonols polymer

**Basic polyphenols analysis**

Avant oxydation      10.4      Après oxydation

Chromatogramme LC-MS - Base peak

HPLC -UV : individual molecules analysis

- Advantages : very well differentiation between polyphenolic compounds.
- limits:
  - expensive
  - Need standard molecules ( if exist)
  - Sample preparation very long

Dosage de Folin-ciocalteu, total Polyphénol content ( no difference between the Pp class, A. Ascorbic)

**IMPORTANT PHYSICAL AND CHEMICAL PROPERTIES OF CAROTENOIDS**

Kimura, M. & Rodriguez-Amaya, D.B. (1999), Brazil:

Quench singlet oxygen      Scavenge or interact with free radicals

Absorb light      CAROTENOIDS      Lipophilic

Easily isomerized And oxidized      Bind to hydrophobic surfaces

**HPLC analysis....examples** (ref: C Mertz and al, 2007)

- Extraction acetone/water/ formic acid
- Qualitative (LC/MS) and quantitative analysis of major compounds
  - ⇒ ellagitannins and anthocyanins (blackberry)
  - ⇒ hydroxycinnamic acids (tree tomato, naranjilla), anthocyanins and kaempferol glycoside (red tree tomato)
- Analysis of minor compounds in blackberries
  - ❖ liq-liq extraction with ethyl acetate
  - ⇒ flavonols ( quercetin and kaempferol glycosides)
  - ⇒ ellagic acid glycosides

Ref: Mertz and al, 7th International Food Data Conference. Sao Paulo, Oct.2007

**POSSIBLE PROTECTIVE MECHANISMS AGAINST CHRONIC DISEASES**

- Quenching of singlet oxygen
- Scavenging of peroxy radicals
- Modulation of carcinogen metabolism
- Inhibition of cell proliferation
- Enhancement of cell differentiation via retinoids
- Stimulation of cell-to-cell communication
- Enhancement of the immune response

**The carotenoids....**

Main reference in the area:

Kimura, M. & Rodriguez-Amaya, D.B. (1999), Brazil

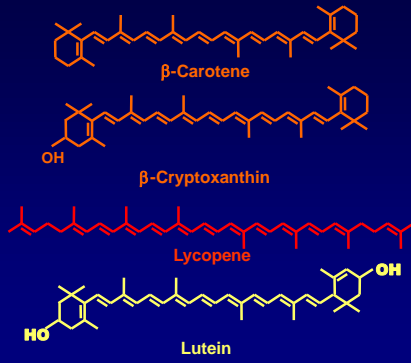
**Carotenoids**

Biology

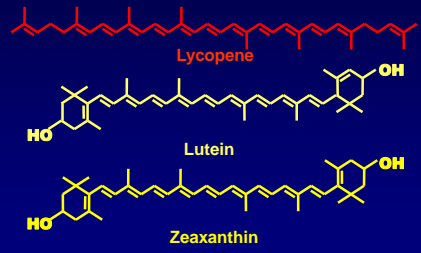
Compound	Pro-Vitamin A status	Chemopreventive activity (Inhibition of C proliferation)	Induction of junctional communication	Relative inhibition of lipid peroxidation
β-Carotene	++++	++++	++++	++
Canthaxanthine	-	++++	++++	+++
Lutein	-	+	+++	+++
Lycopene	-	+(+)	++	+++
Vit E	-	+	±	++++
Retinoic acid	++++	++	+++	ND
Acyclorethinoic acid	-	+(+)	+	ND

Kimura, M. & Rodriguez-Amaya, D.B. (1999), Brazil:

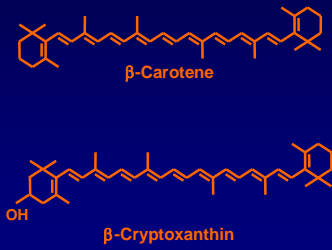
## PRINCIPAL CAROTENOIDS IN FOODS



## CAROTENOIDS CONSIDERED IMPORTANT TO HUMAN HEALTH



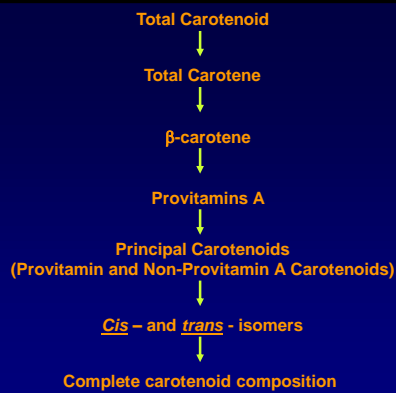
## CAROTENOIDS CONSIDERED IMPORTANT TO HUMAN HEALTH



The and Antioxydant value

And

bioavailability approach



## EVOLUTION OF CAROTENOID ANALYSIS

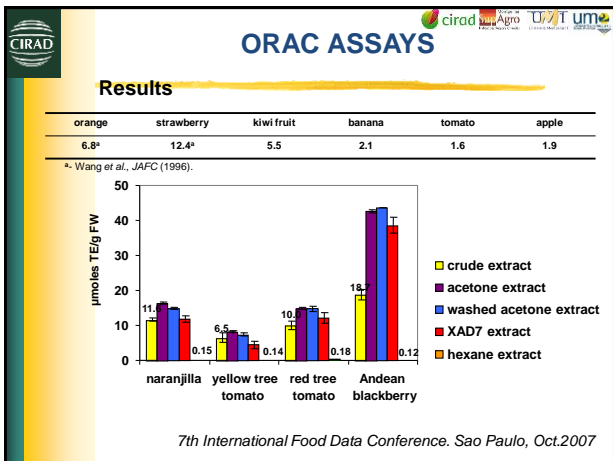
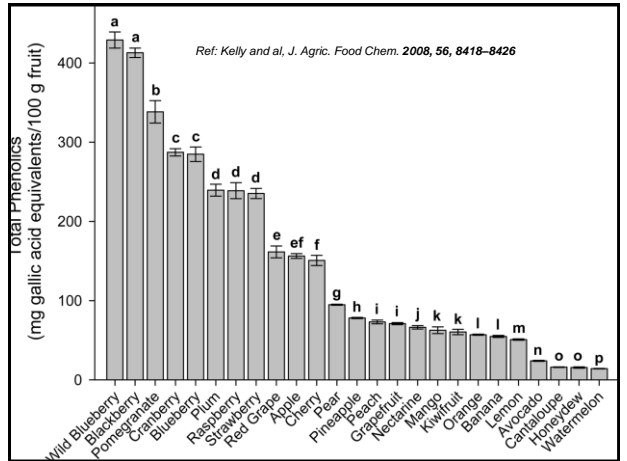
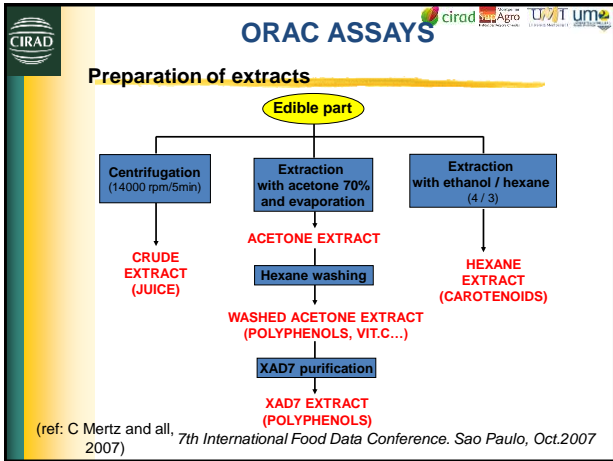
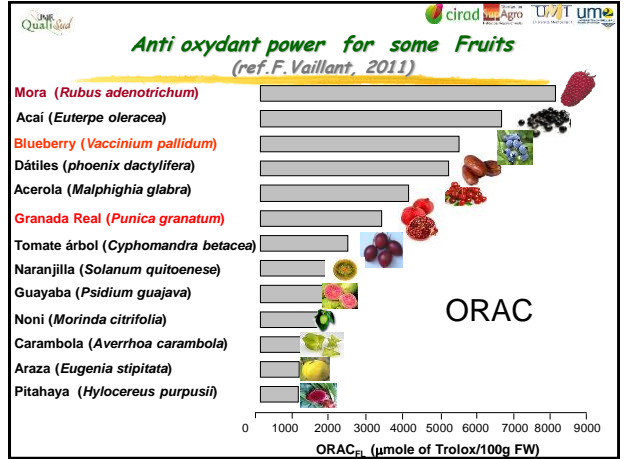
The Antioxydant value

## The Antioxydant values and general analysis to be done in indigenous fruits

(ref: F. Vaillant/ Cirad)

- (1) By hplc
- (2) Total anthocyanin
- (3) Betalain by hplc
- (4) Total betalains
- (5) Carotenoids by hplc
- (6) Total carotenoids
- (7) Vitamin C by hplc
- (8) Polysaccharides analysis
- (9) Total Dietary fibres
- (10) Polyphenol by hplc

- (1) Total polyphenols by Folin
- (2) Antioxidant by FTC value
- (3) Antioxidant by TOSC value
- (4) LC-MS determination of compounds with antioxidant capacity by TOSC
- (5) Antioxidant ORAC FL value
- (6) Antioxidant DPPH value
- (7) Antioxidant FRAP value
- (8) Antioxidant TEAC value
- (9) *In-vivo* bioavailability of the antioxidant capacity, TOSC on human blood plasma
- (10) *In-vivo* bioavailability of the antioxidant capacity ORAC on human blood plasma
- (11) Metabolomic evaluation



## the bioavailability approach

UNE QualiQual

cirad Agro UVT um

## The bioavailability approach

- \*Caco CELL in laboratory  
With articia estomago
- \* In vivo studies ( rates....)
- \* in vivo studies ( human....)

UNE QualiQual

cirad Agro UVT um

UNE QualiQual

cirad Agro UVT um

Fig. 3 Schematic diagram of a multiple-step *in vitro* digestion model to simulate the whole of the GI tract.

40. J. Food Funct., 2010, 1, 32-59

UNE QualiQual

cirad Agro UVT um

### CACO2 approach .... (ref Wikipedia/ internet)

Caco-2 cells are most commonly used not as individual cells, but as a confluent monolayer on a cell culture insert filter

When cultured in this format, the cells differentiate to form a polarized epithelial cell monolayer that provides a physical and biochemical barrier to the passage of ions and small molecules.

The Caco-2 monolayer is widely used across the pharmaceutical industry as an in vitro model of the human small intestinal mucosa to predict the absorption of orally administered drugs.



The correlation between the in vitro apparent permeability across Caco-2 monolayers and the in vivo fraction absorbed is well established.!

**BIOAVAILABILITY OF FLAVONOIDS**  
(Reference: E.Rock, INRA France)

UNE QualiQual

cirad Agro UVT um

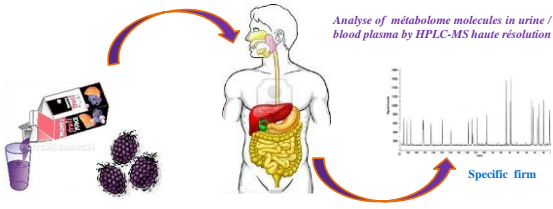
## a new way of nutritional evaluation of fruits metabolites: THE METAGENOMIC


 AXE PRIORITAIRE: ALIMENTATION ACCESSIBLE ET DE QUALITE - 

**Process impact on métabolites de phytomicronutriments with human nutritional interest**

Métabolome = all the metabolites coming from estomago and coming from food metabolism (pH effect...)



**Metabolomic concept**



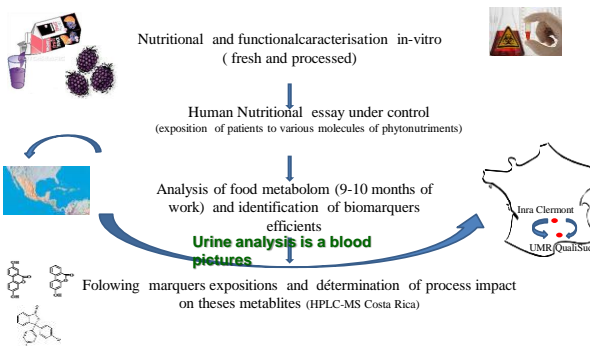
Analyse of métabolome molecules in urine / blood plasma by HPLC-MS haute résolution

Specific firm

✓ Comment sont reflétées sur le métabolomes des variations de qualité de l'aliment ?  
 ✓ Quel impact potentiel sur la santé dans le cadre d'une nutrition préventive ?


 AXE PRIORITAIRE: ALIMENTATION ACCESSIBLE ET DE QUALITE - 

**Méthodology**



Nutritional and functional characterisation in-vitro (fresh and processed)


Human Nutritional essay under control (exposition of patients to various molecules of phytonutriments)


Analysis of food metabolom (9-10 months of work) and identification of biomarkers efficientes

**Urine analysis is a blood pictures**

Following marquers expositions and détermination of process impact on theses metablités (HPLC-MS Costa Rica)

Inra Clermont  
 UMR QualiSud



UMR QualiSud

**THANKS**