La Métabolomique :

Une approche sophistiquée globale du vivant

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OPINION

2020 visions

For the first issue of the new decade, *Nature* asked a selection of leading researchers and policy-makers where their fields will be ten years from now. We invited them to identify the key questions their disciplines face, the major roadblocks and the pressing next steps. Visit go.nature.com/htW8uM to respond and to add your vision.

Microbiome Energy Synthetic

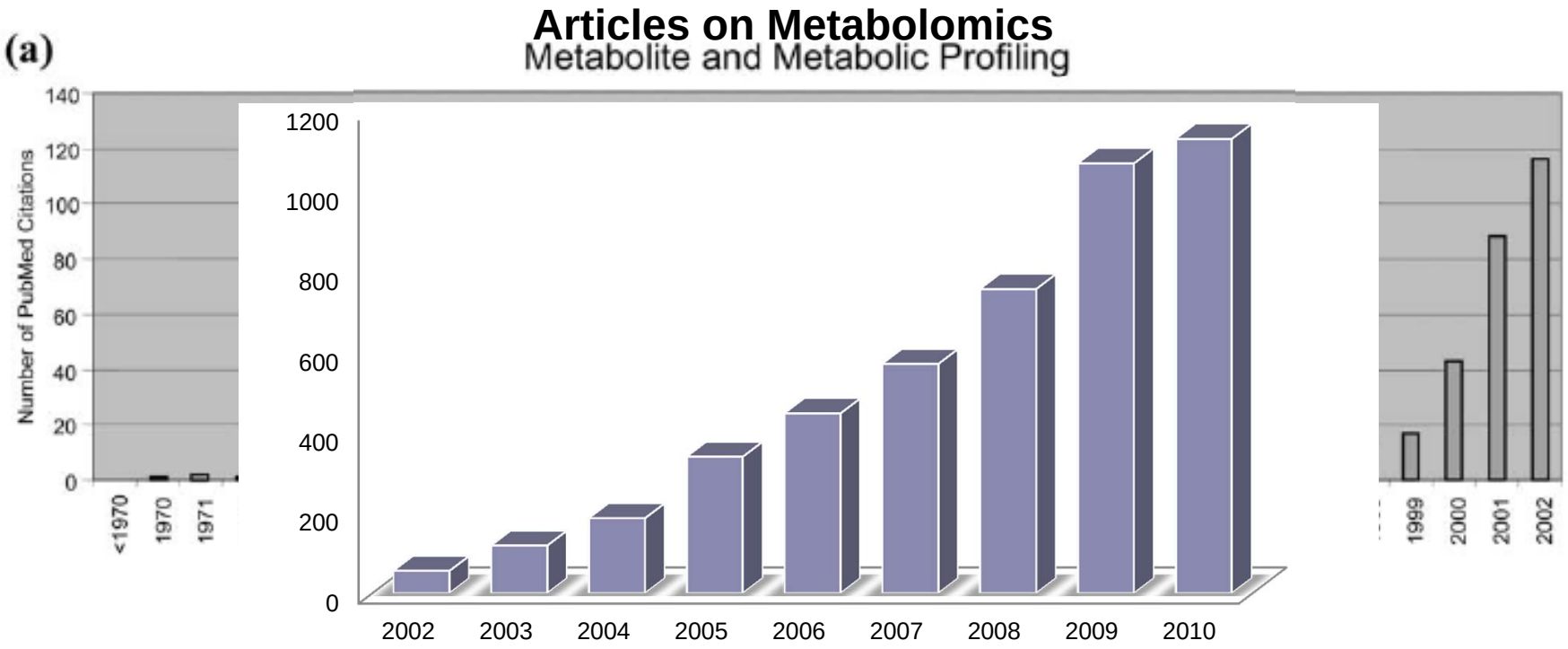
Perso Metabolomics

medicine Astronomy palaeontology

Drug discovery Chemistry

Lasers

HISTORY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Number of PubMed Citations
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120
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Articles on Metabolomics
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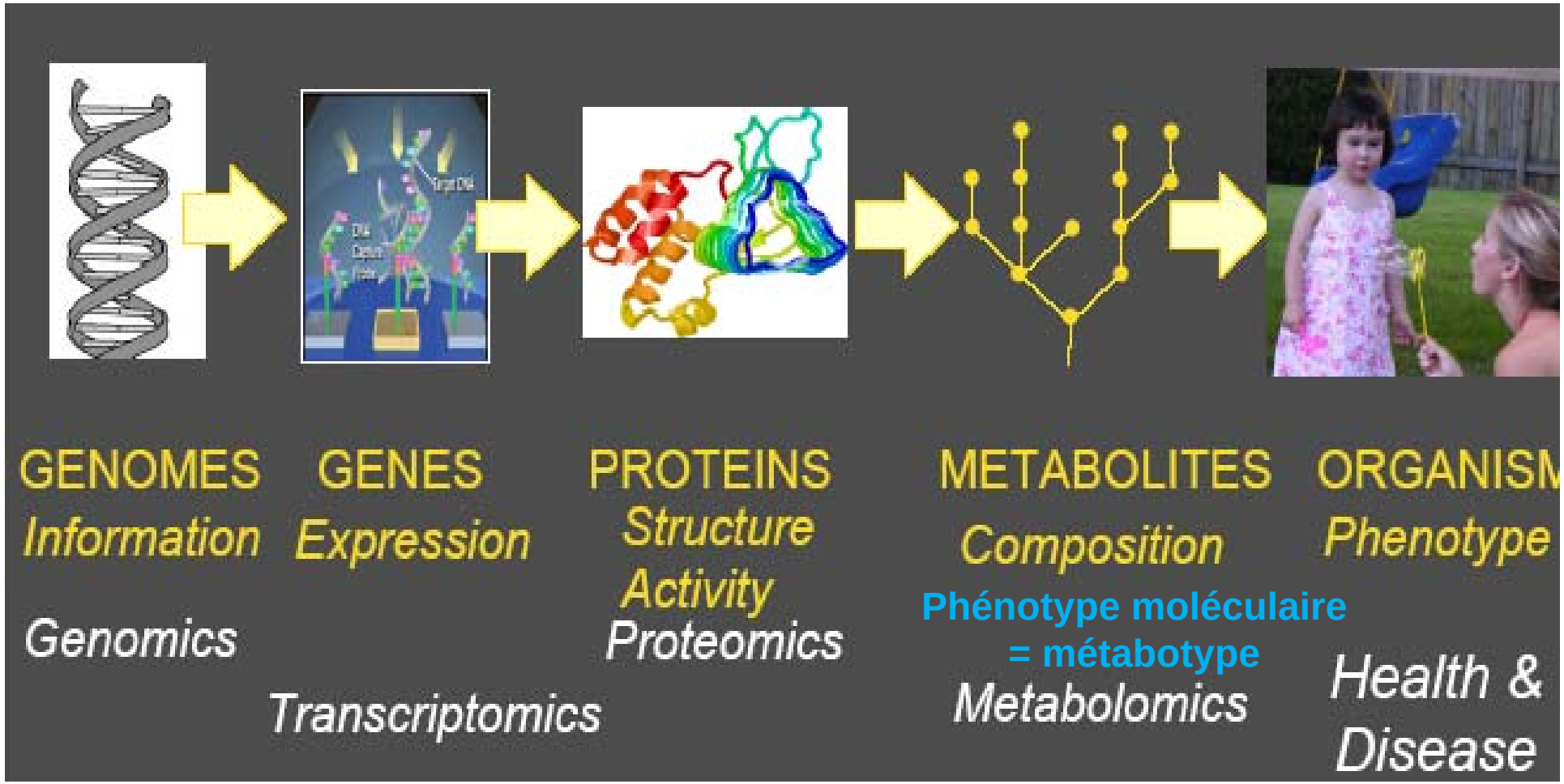
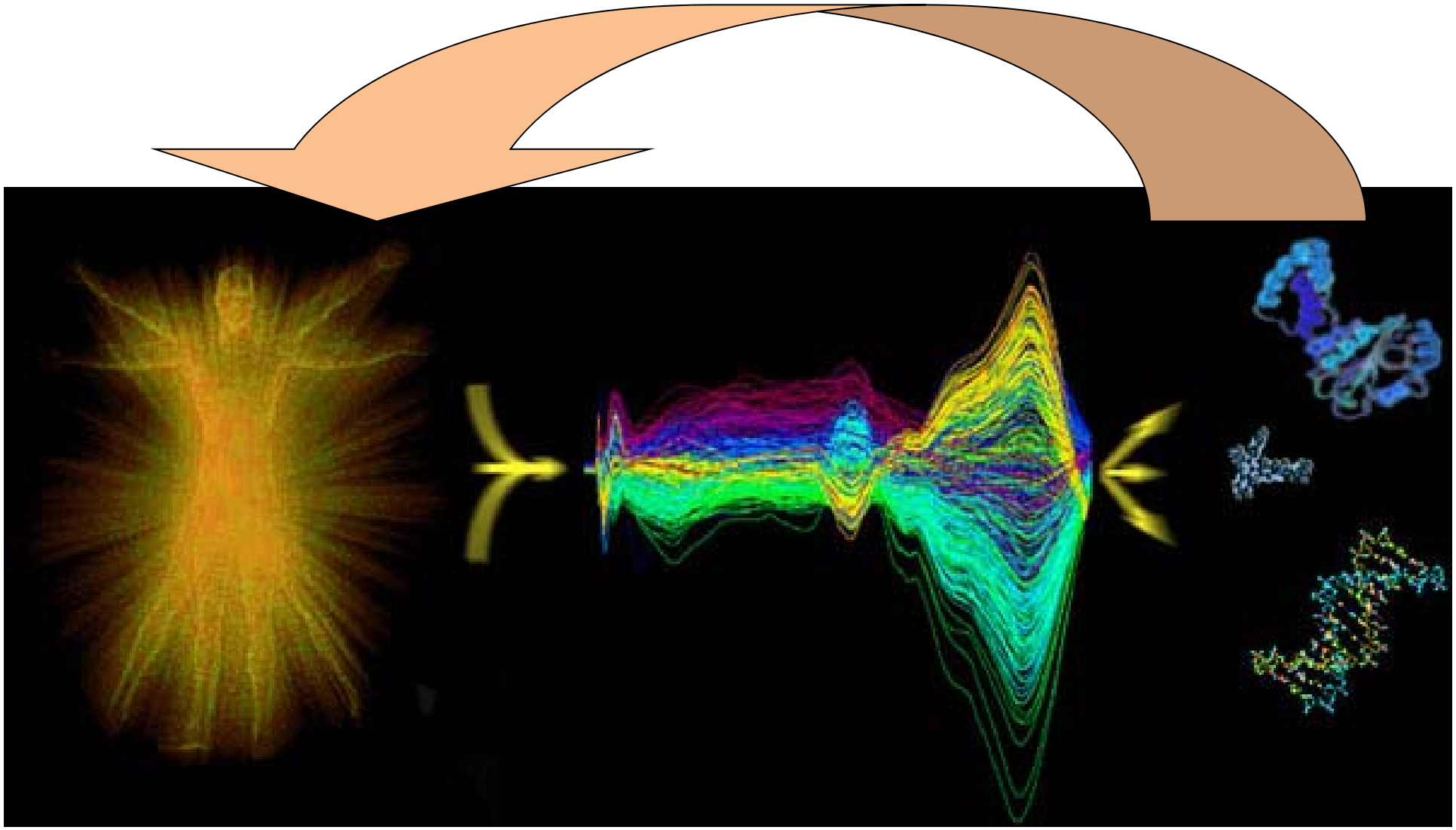


« Le métabolome représente l'ultime réponse d'un organisme à une altération génétique, une pathologie, une exposition à un toxique ou toute cause environnementale » (E. Ezan)



http://masspec.scripps.edu/index.php

Métabolomique/métabonomique

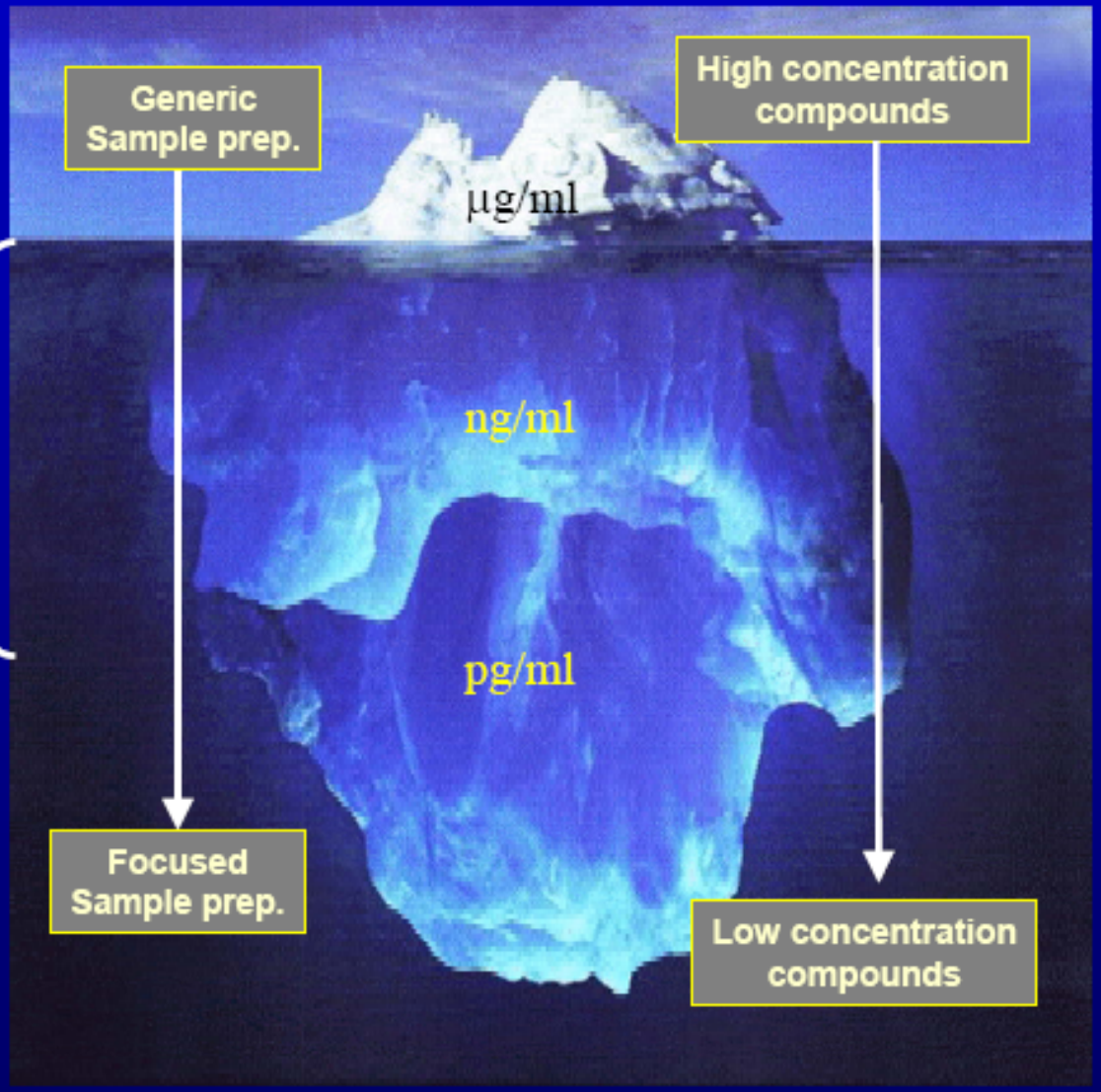
• donne la description détaillée de la composition en métabolites des biofluides, des cellules et des tissus

• détecte les perturbations (stress, physiopathologie) d'entités métaboliques spécifiques et les relie à des processus, voies ou mécanismes biochimiques utilisables pour:

- fonction de gènes
- études toxicologiques/pharmacologiques
- stress environementaux
- diagnostic / prévention des maladies
- nutrition

Approche globale: réponse analytique pour aborder la complexité

Profils des liquides biologiques : Le challenge de l'empreinte métabolique



NMR

Mass Spectrometry

CUSTOM

Homeostasis 'Housekeeping'

Organic Acids

Lipids

Amino Acids

Nucleotides

Steroids

Eicosanoids

Neurotransmitters

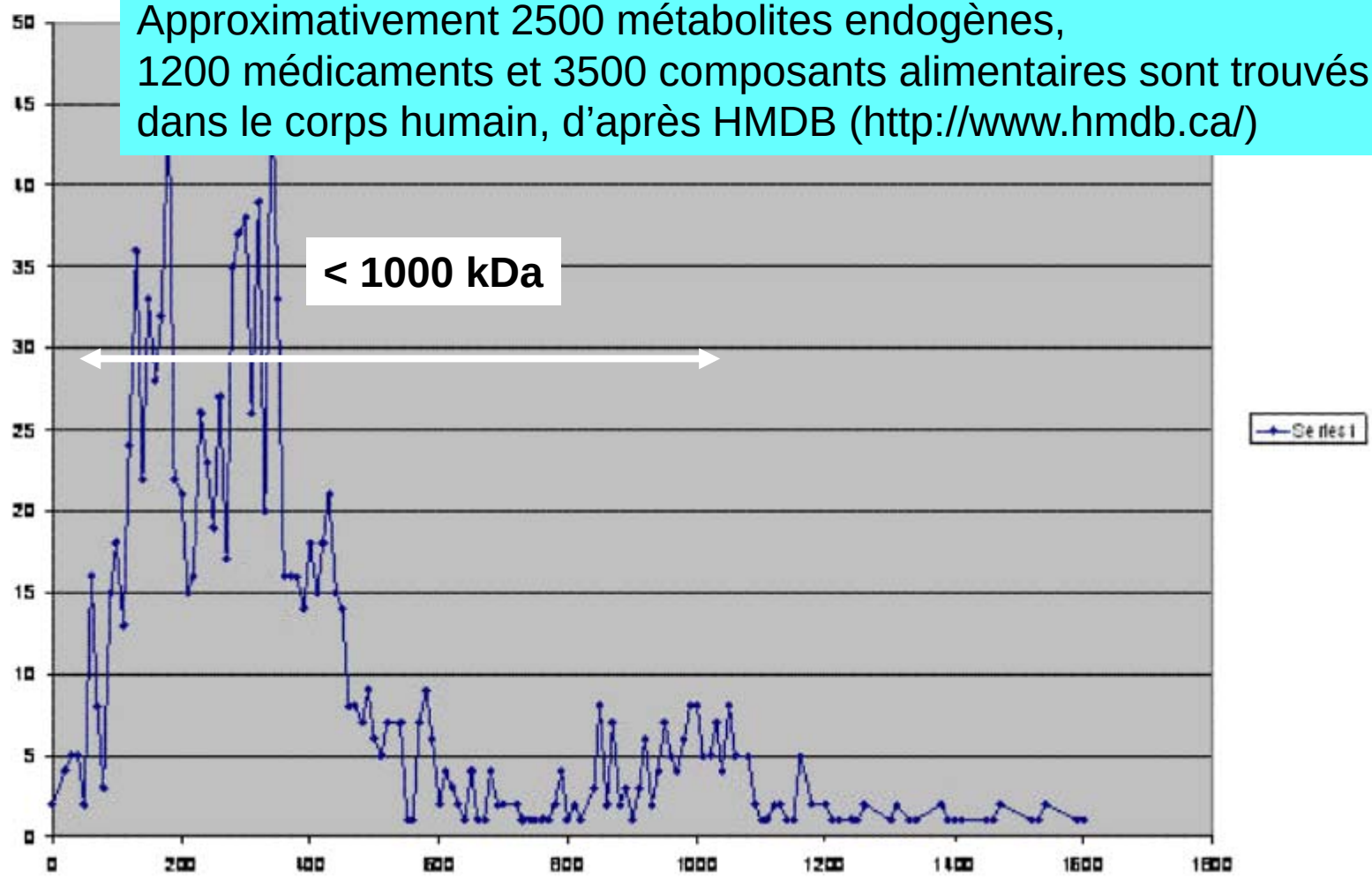
Peptides

Trace elements

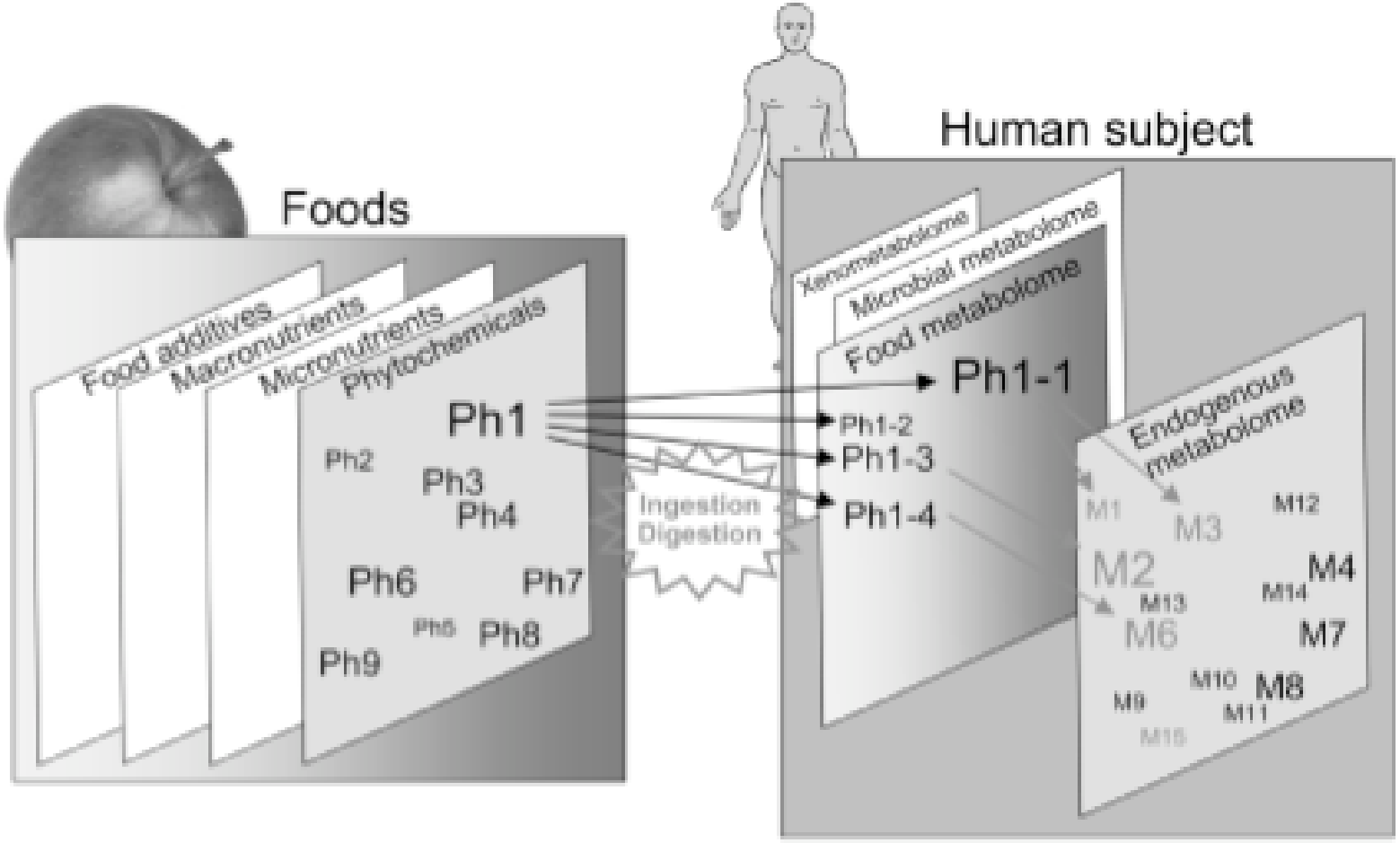
Comprehensive bioanalysis requires multiple techniques to cover wide concentration range

Mass Distributions of Human Metabolome

Approximativement 2500 métabolites endogènes,
1200 médicaments et 3500 composants alimentaires sont trouvés dans le corps humain, d'après HMDB (http://www.hmdb.ca/)



Interaction entre alimentation et individu



Manach et al., MNFR 2009

Echantillons

↓

Traitement → **Acquisition des Données (1 pic = 1 variable)**

↓

Réduction des données

↓

Analyse statistique Multivariée (espace 2D ou 3D) (chimiometrie) **(PCA, PLS-DA,…)**

↓

Interprétation

↙ ↓ ↘

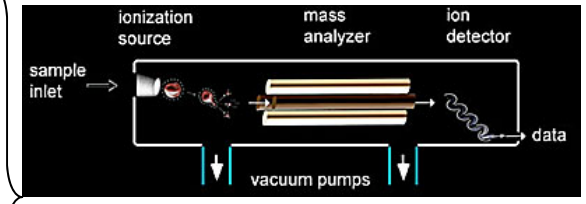
Comparaison de groupes: appartenance aux classes ↔ **Diagnostic individuel: prédiction** **Identification biomarqueurs discriminants**

Plate-formes métabolomique

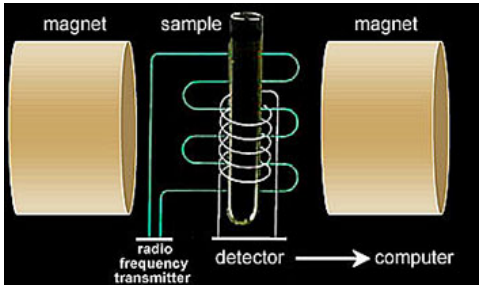
Systèmes de séparation chromatographique

Détecteur

+	-
Haute résolution	Volatilité (dérivation)
Applicable nbreux métabolites	Moins résolutif CPG **(UHPLC~CPG)**



2000 signaux après déconvolution



500 signaux après déconvolution

La métabolomique: quelle infrastructure….

Chimistes

Machine(s)

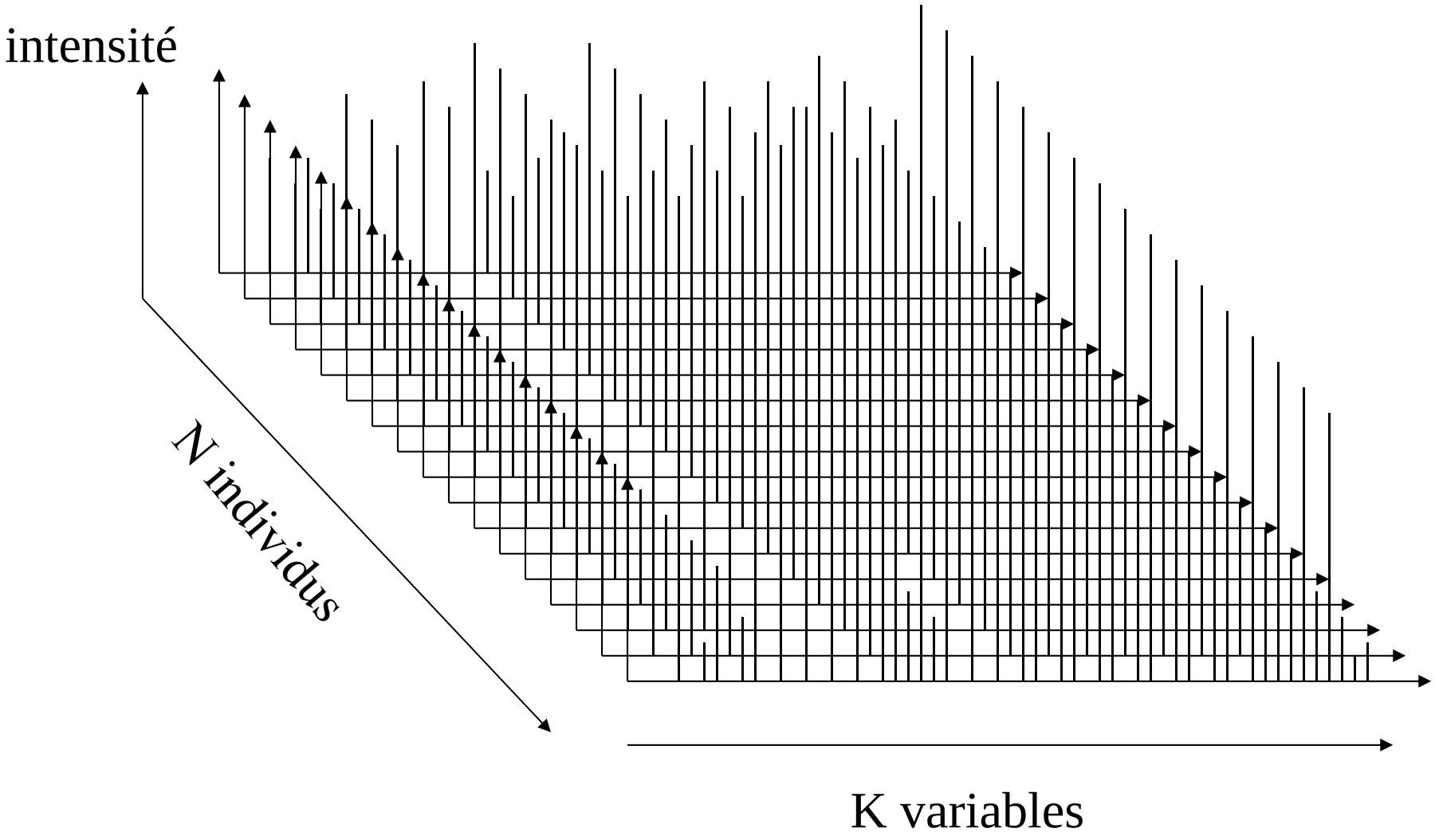
Biologistes

Ingénieurs
Chercheurs
Cliniciens
Technicien

réseau

Bioinformatique

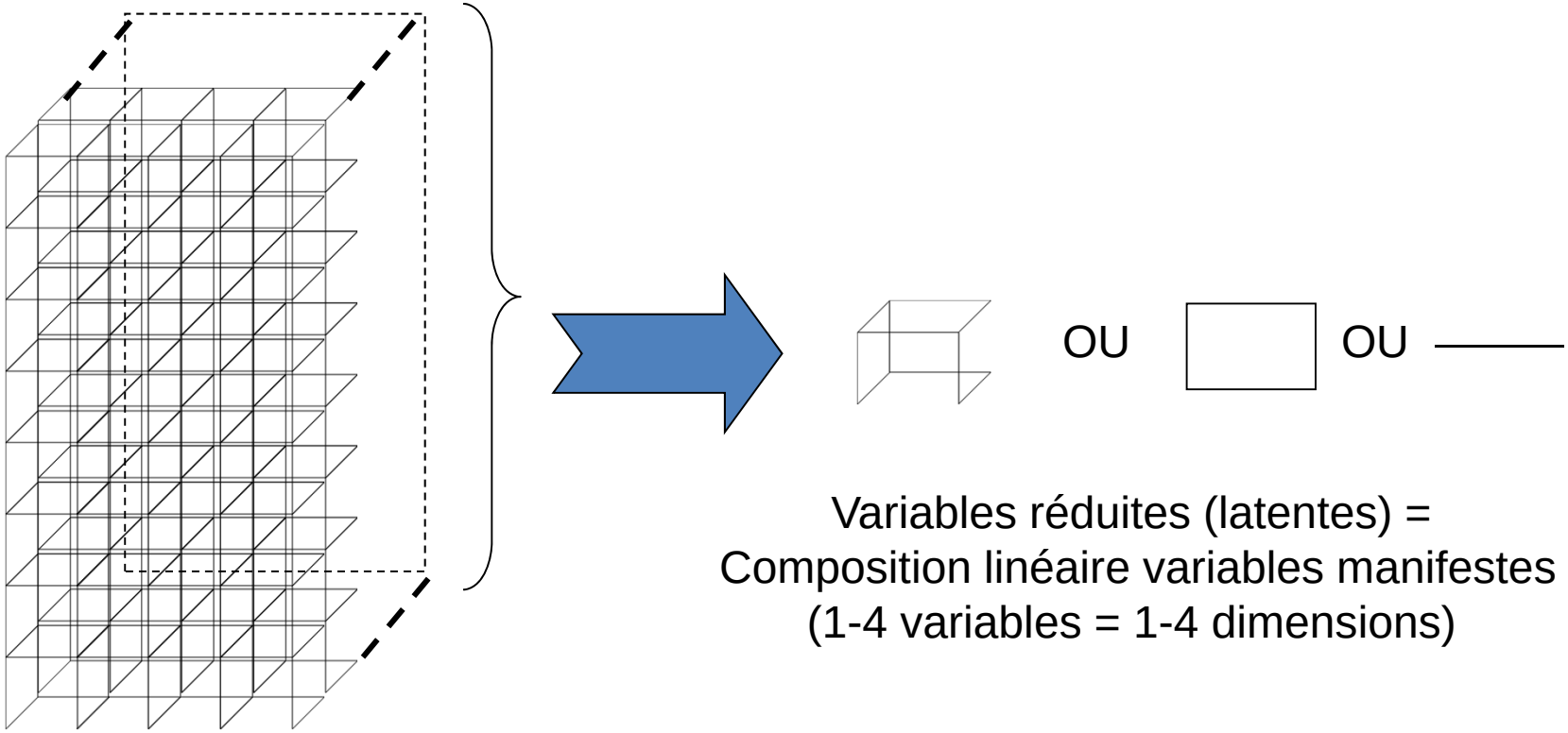
Statisticiens


intensité
N individus
K variables

1 variable = 1 dimension

1000 variables = 1000 dimensions

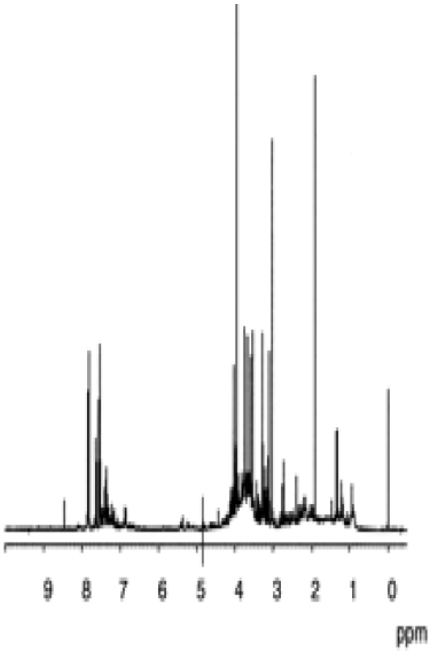
BUT = REDUIRE SANS PERTE D'INFORMATION

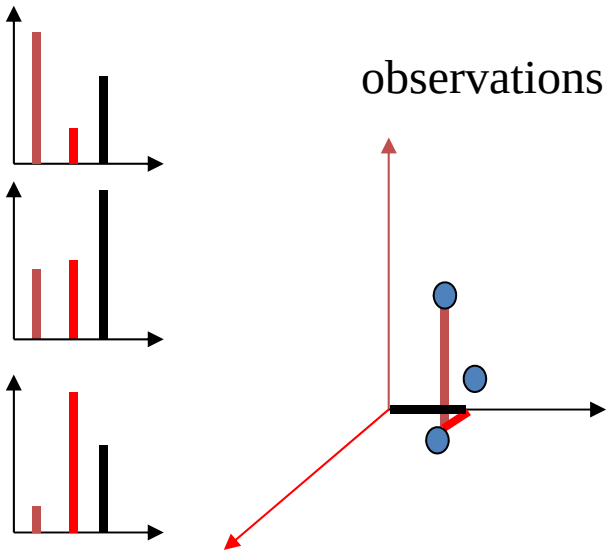


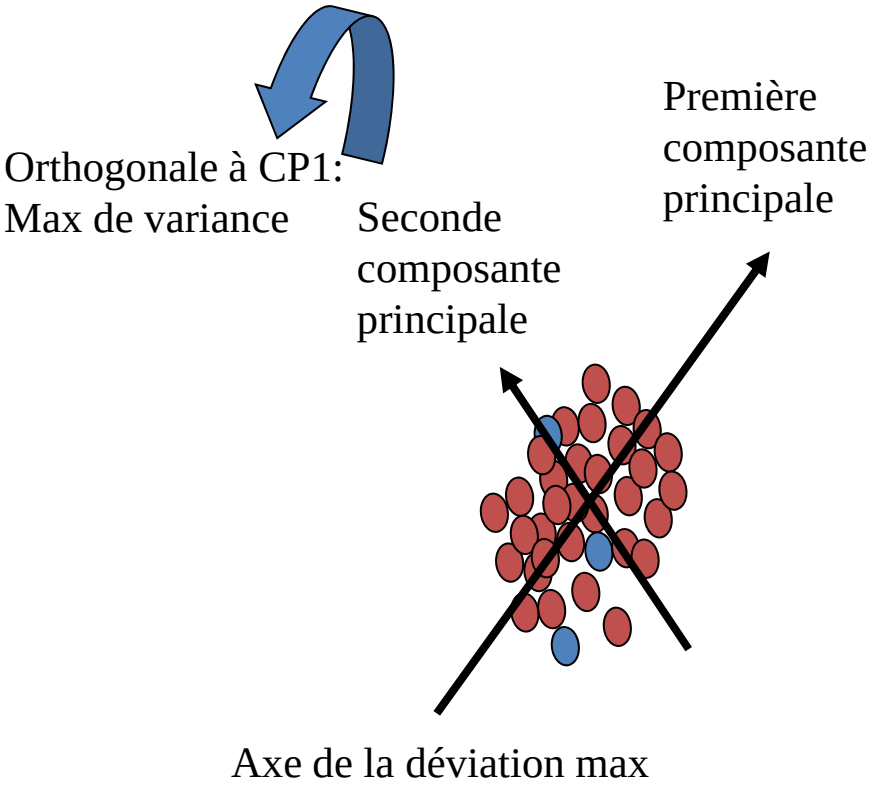
Variables réduites (latentes) =
Composition linéaire variables manifestes
(1-4 variables = 1-4 dimensions)

K variables manifestes= espace k dimensions
(Hyperespace)

Réduction et traitement des données

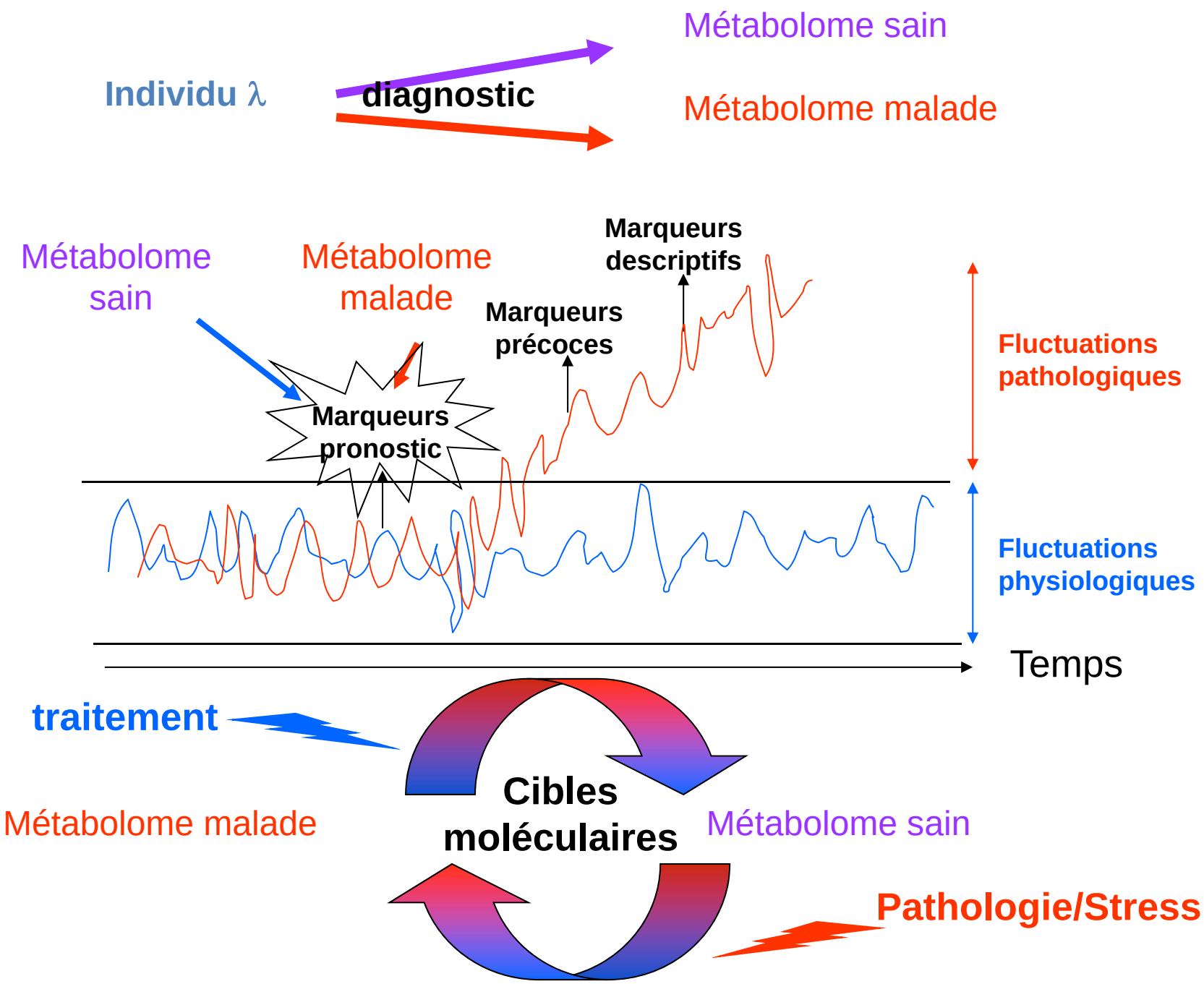






Analyses en composantes principales,
Analyses discriminantes, etc…

APPLICATIONS


Métabolome sain
Individu λ
diagnostic
Métabolome malade
Métabolome sain
Métabolome malade
Marqueurs descriptifs
Marqueurs précoces
Marqueurs pronostic
Fluctuations pathologiques
Fluctuations physiologiques
Temps
traitement
Métabolome malade
Cibles moléculaires
Métabolome sain
Pathologie/Stress

Mesure des trajectoires et distances métaboliques

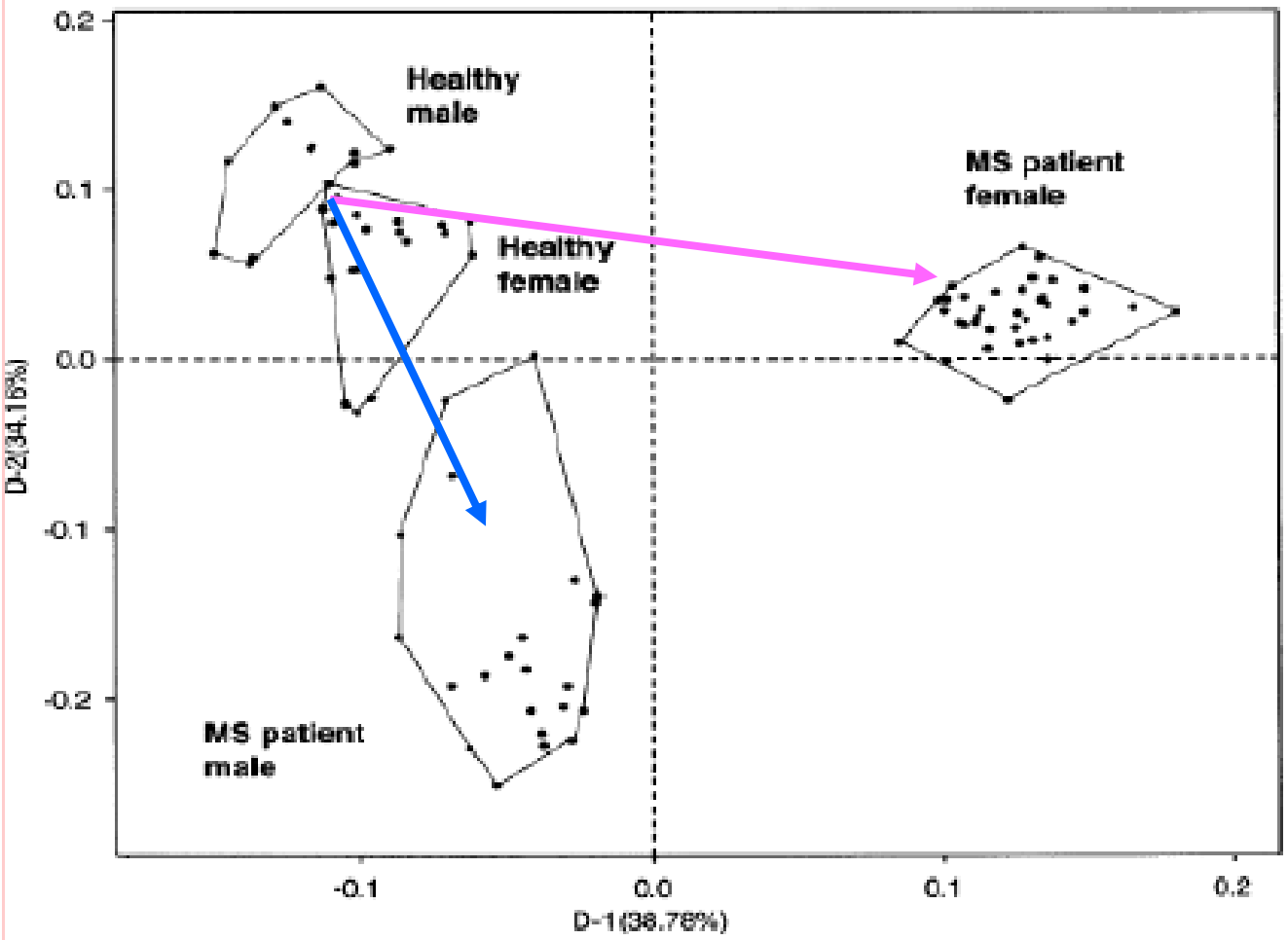


Fig. 2.

Homme : effets de régimes avec des fruits

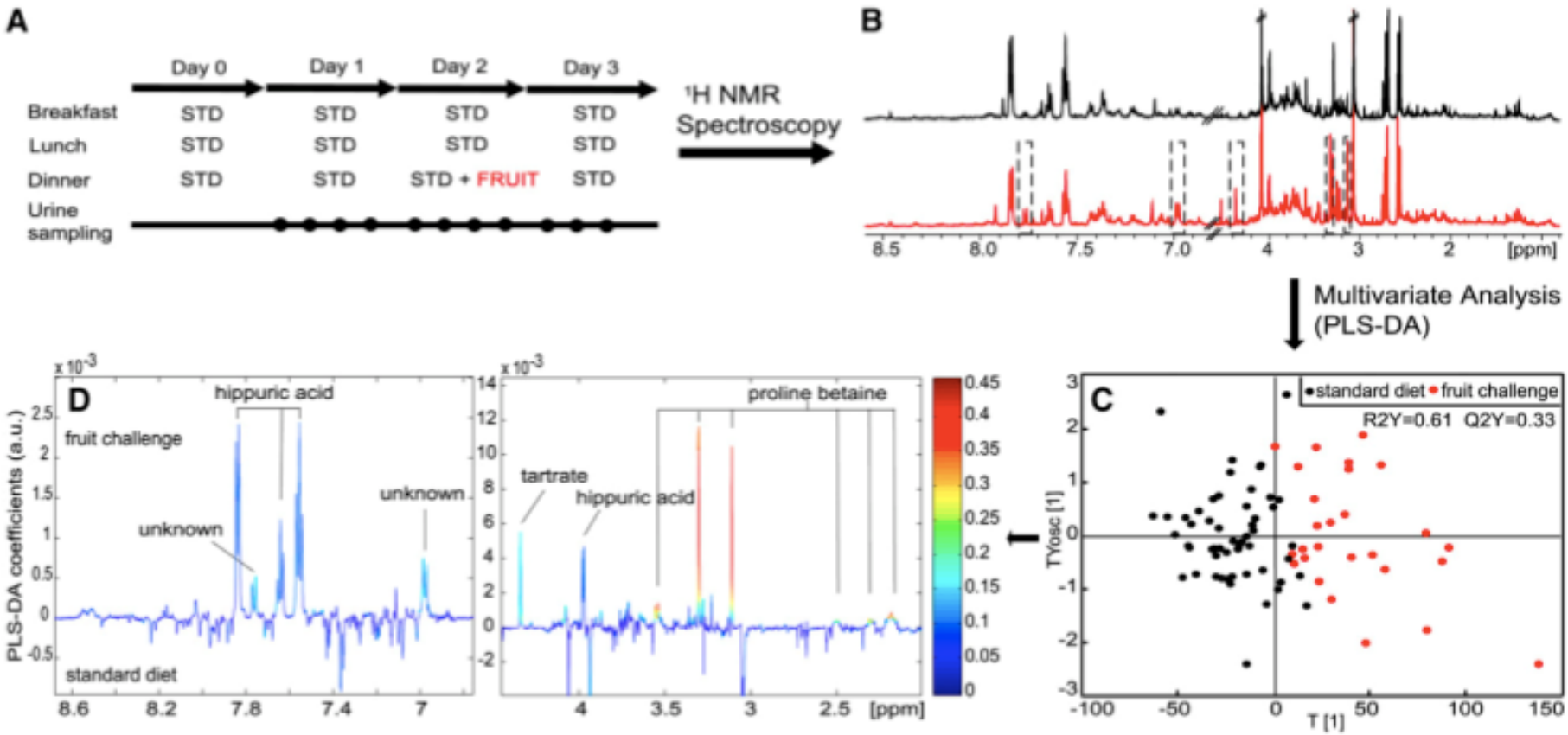
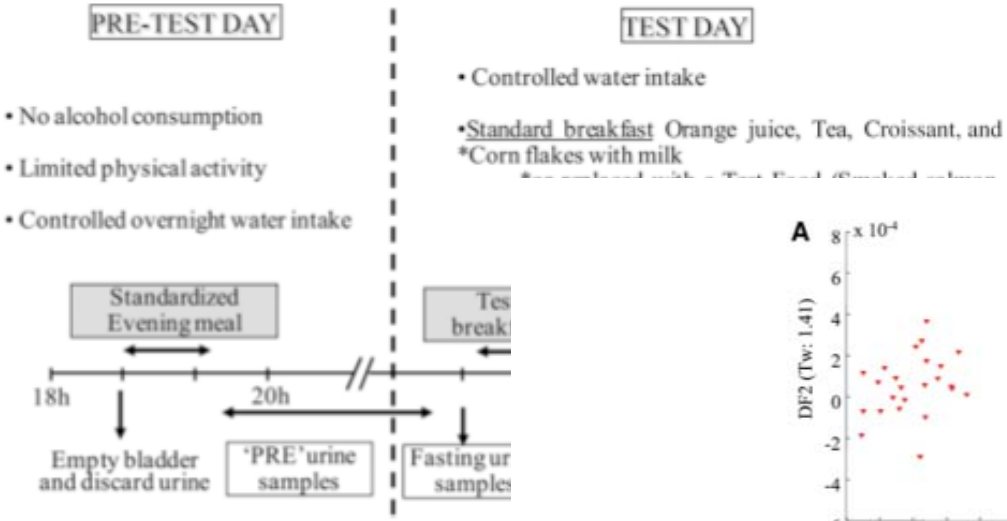


FIGURE 1. Identification of putative biomarkers by using metabolite profiling and multivariate analysis. A: Study design for the dietary intervention study (n = 8). B: Representative ^{1}H nuclear magnetic resonance (NMR) spectra of urine specimens in response to fruit consumption (red) compared with the standard (STD) meal (black). Apparent differences are highlighted (dashed rectangles). C: Partial least-squares discriminant analysis (PLS-DA) scores plot of urine specimens 0–24 h after fruit challenge, which shows a clear separation of the fruit and STD meals. All urine specimens from the morning of day 1 to the

Walsh et al., Am J Clin Nutr. 2010

Homme : signature des aliments



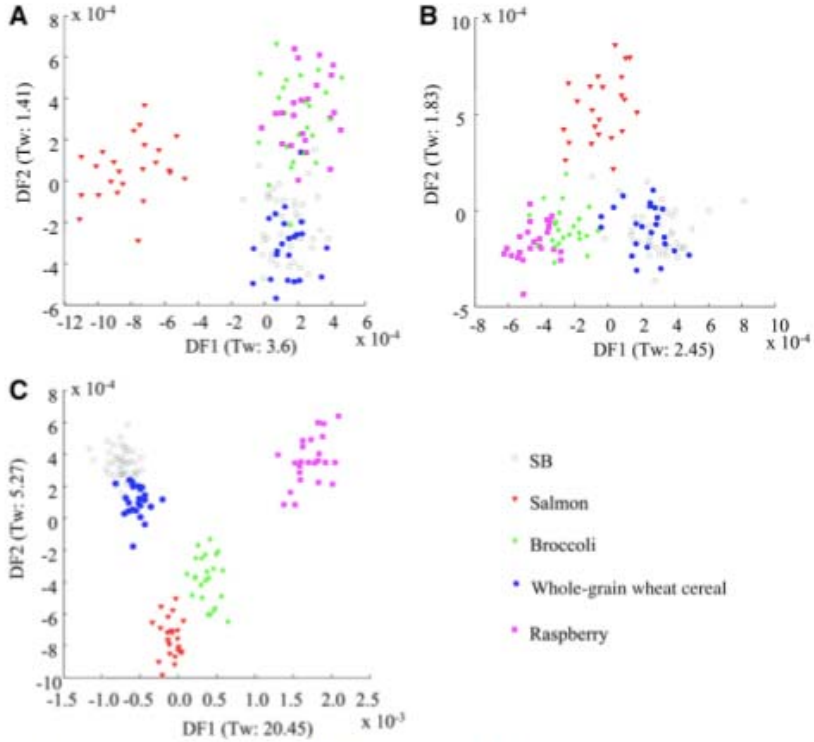


FIGURE 2. Modeling characteristics of different types of metabolite fingerprinting data comparing urine samples after consumption of the SB and test breakfasts containing each of 4 specific foods. Principal components–linear discriminant analysis was applied to metabolite fingerprinting data (*m/z*: 100–510)

Llyod et al., Am J Clin Nutr. 2011

Homme : signature des aliments

TABLE 4
Identity of metabolites in explanatory mass bins associated with the consumption of specific foods consumed in test breakfasts obt mass analysis and tandem mass spectrometry[1]

Nominal mass	Major signal in FT-ICR-MS	Putative metabolite (1 ppm)
Potential salmon biomarker signals		
241	241.12964	Anserine
242	242.13302	Anserine
192	192.07427	1- and 3-Methylhistidine
208	208.04825	1- and 3-Methylhistidine
279	279.08567	Anserine
221	221.06445	Unknown
151	151.14409	Trimethylamine-*N*-oxide
Potential raspberry biomarker signals		
259	259.02808	Caffeoyl sulfate
211	211.00160	Ascorbate
383	383.04419	Methyl-epicatechin sulfate
183	Several masses	Unknown
176	176.02826	Ascorbate
341	341.12405	Unknown glucuronide
194	194.04603	3-Hydroxyhippuric acid
447	447.09330	Naringenin glucuronide
Potential broccoli biomarker signals		
211	211.00160	Ascorbate
176	176.02826	Ascorbate
135	135.02996	Tetronic acids
103	103.04010	Tetronic acid derivative
165	165.04051	L-Xylonate/L-lyxonate
104	104.04344	Tetronic acid derivative
121	121.05068	Threitol/erythritol
447	447.09330	Naringenin glucuronide
477	477.10421	Hesperitin glucuronide plus other glucuronides

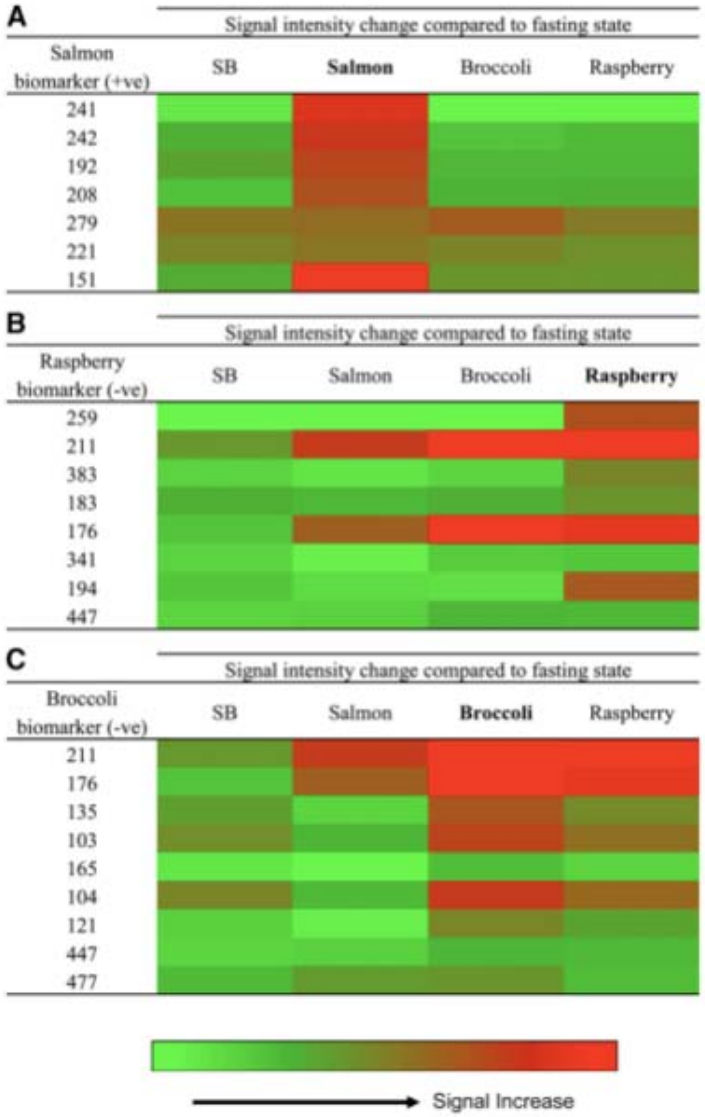
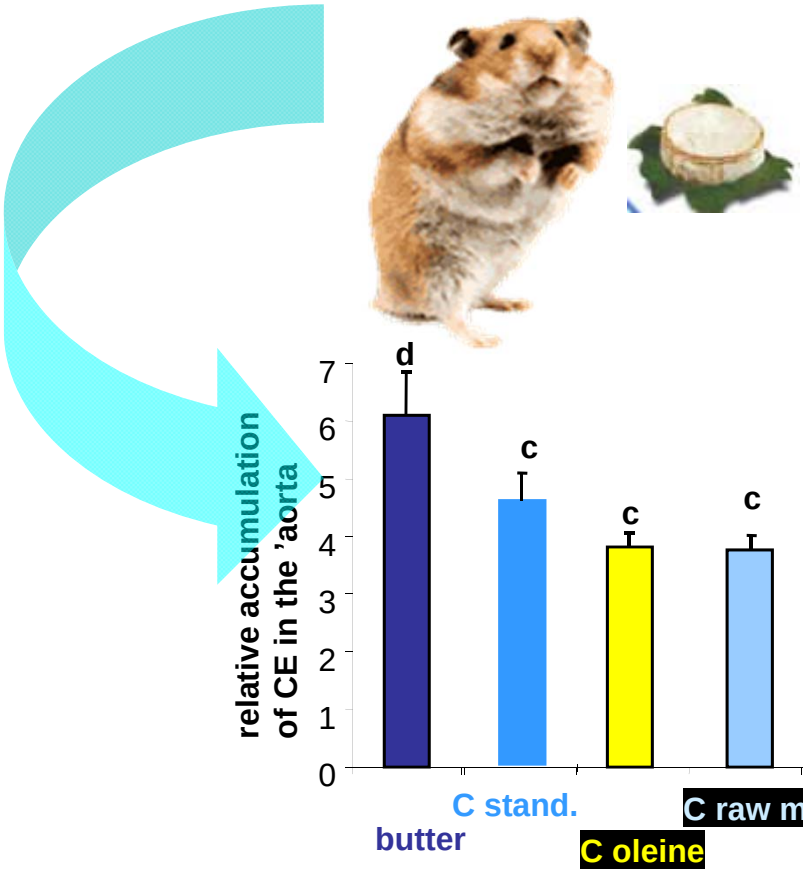
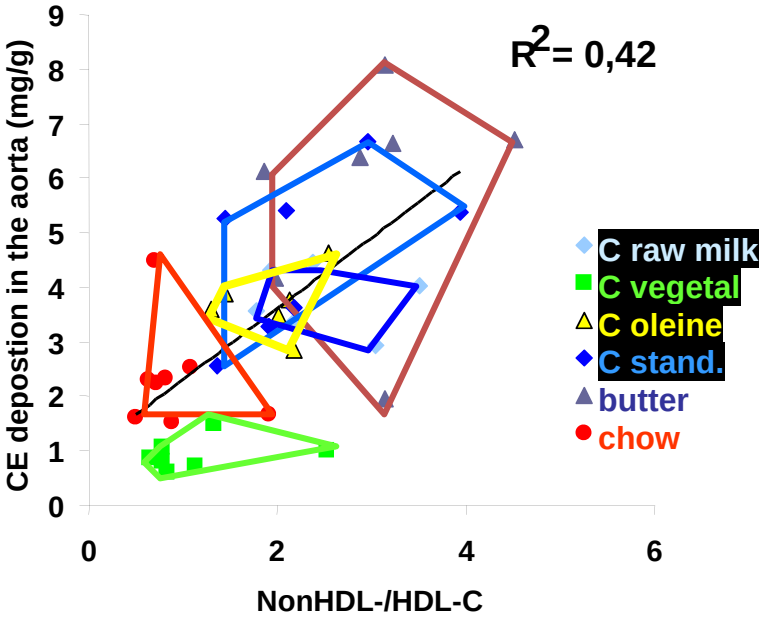
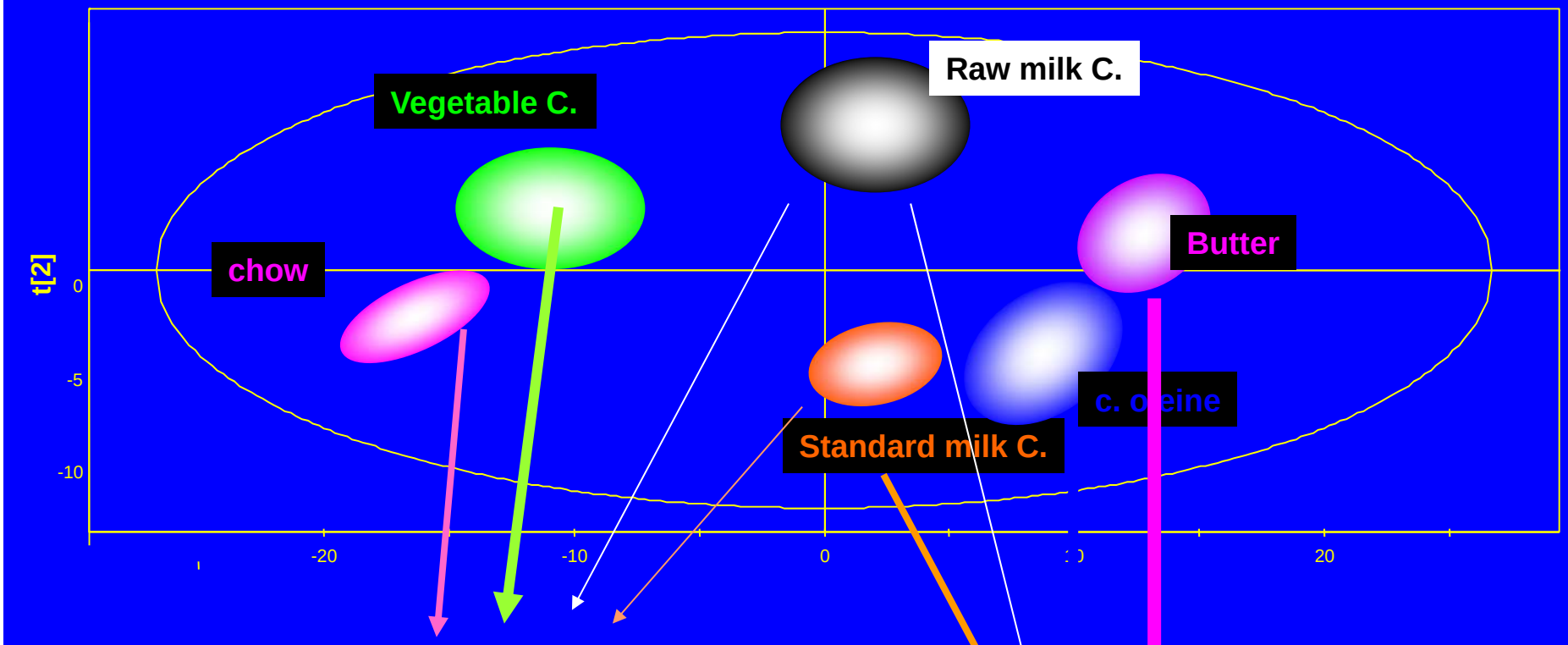


FIGURE 3. Changes in signal intensity in urinary potential biomarkers after consumption of each of 3 test foods and of the SB. Heat map repre-

Llyod et al., Am J Clin Nutr. 2011


X 12 weeks
relative accumulation of CE in the 'aorta
0
1
2
3
4
5
6
7
d
c
c
c
b
a
butter
C stand.
C oleine
C raw milk
chow
C vegetal


R^2= 0,42
CE depostion in the aorta (mg/g)
0
1
2
3
4
5
6
7
8
9
0
2
4
6
NonHDL-/HDL-C
C raw milk
C vegetal
C oleine
C stand.
butter
chow



- Amino-acid metabolism
- Low oxidative stress
- blood lipid clearance
- Muscle energy metabolism
- Intestinal microbiota metabolism

- Atherogenic particles (VLDL > LDL)
- Inflammation

Low atherogenic outcome

High atherogenic outcome

Maïs : variations (variétés, années, terroirs)

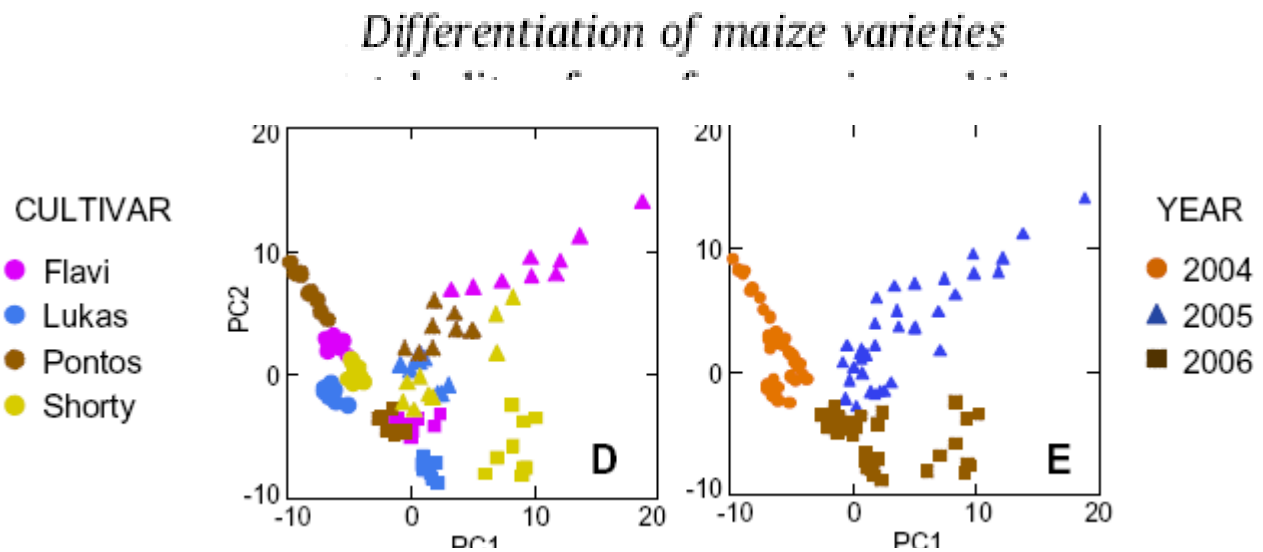
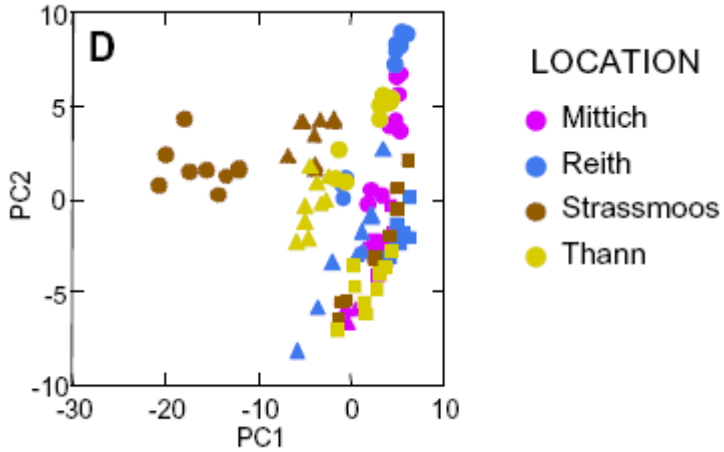


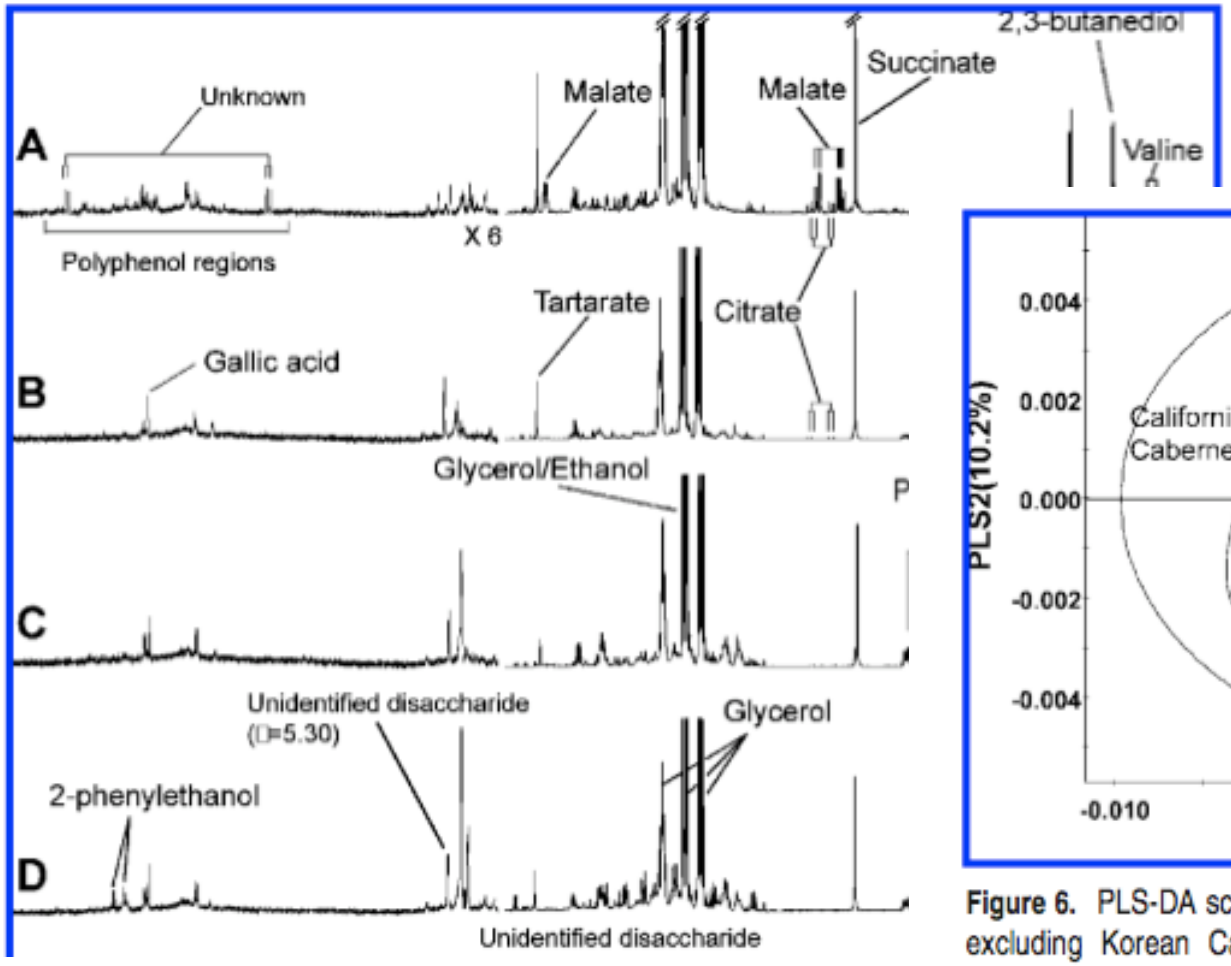
Fig. 1. Principal components analysis of metabolite profiling data from fractions I to IV in growing seasons 2004 (A), 2005 (B), 2006 (C) and from combined data of 2004–2006 (D and E) at farming location Frankendorf, Bavaria.



Davies et al. 2010

Fig. 2. Principal components analysis of metabolite profiling data from fractions I to IV of cultivar Amadeo in growing seasons 2004 (A), 2005 (B), 2006 (C) and combined 2004–2006 (D) at the four locations Mittich, Reith, Strassmoos and Thann.

Vins: effets des variétés et terroirs



1H NMR spectra of wines
B, C, D : Cabernet Sauvignon F, Cal, Aus.

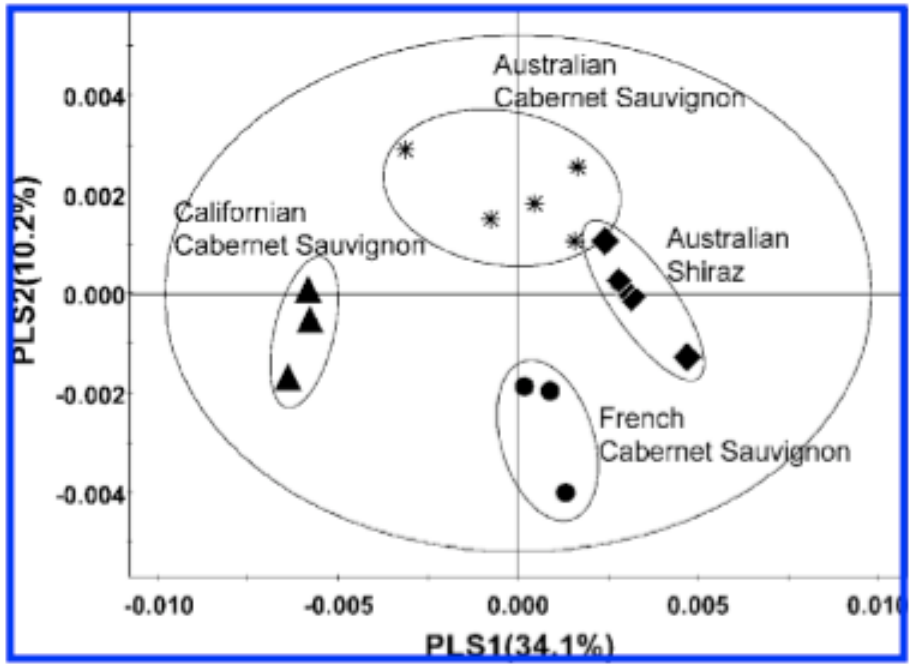
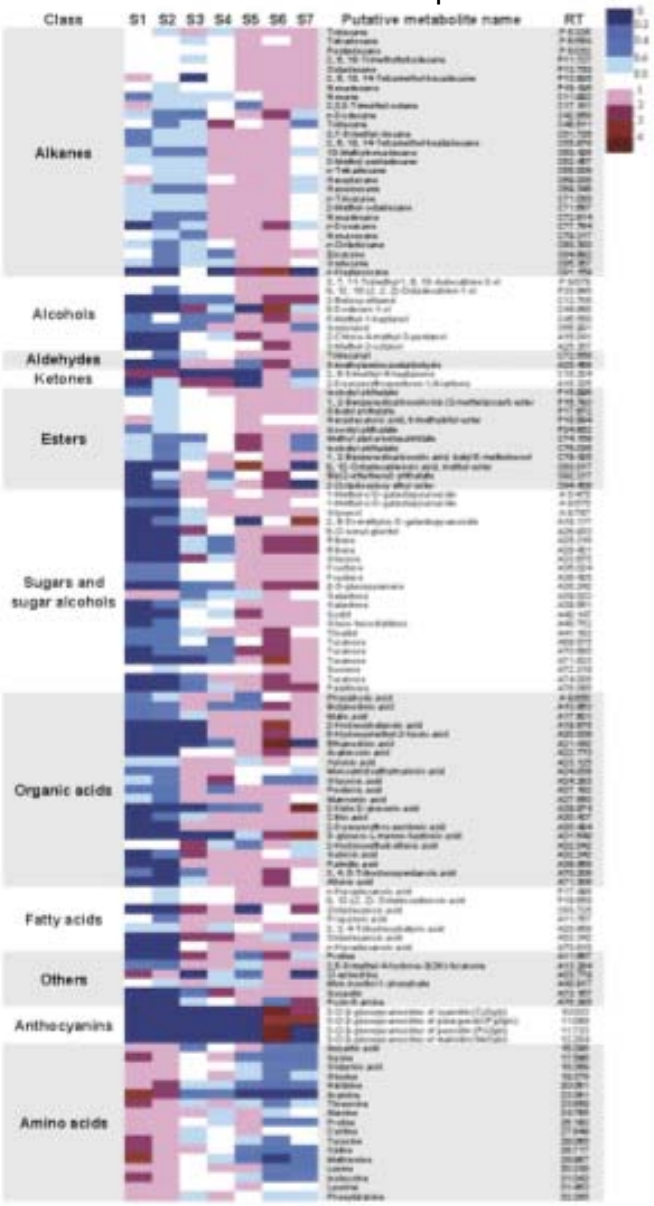


Figure 6. PLS-DA score plot derived from the ^{1}H NMR spectra of wines excluding Korean Campbell Early wines. The plot shows the clear discrimination of Australian Shiraz (square) and French (circle), Californian (triangle), and Australian (star) Cabernet Sauvignon wines.

Son et al, JAFC 2008

Fraises: effets des stades de développement

Metabolic profiling of strawberry fruit
par GC-MS



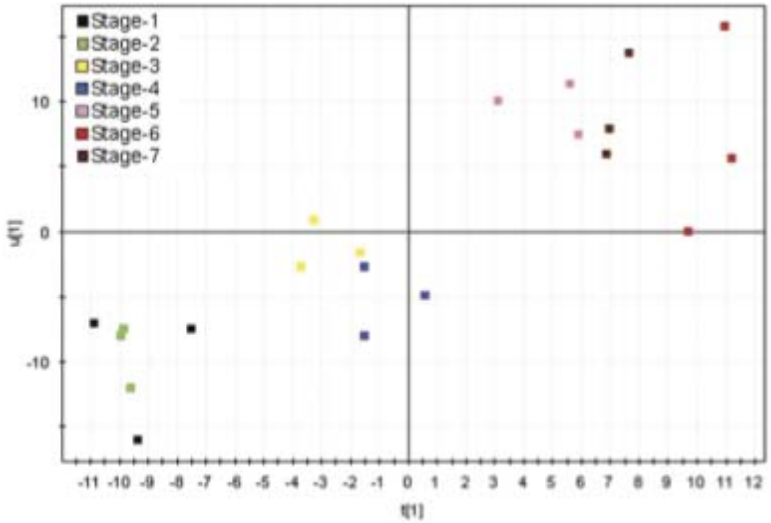
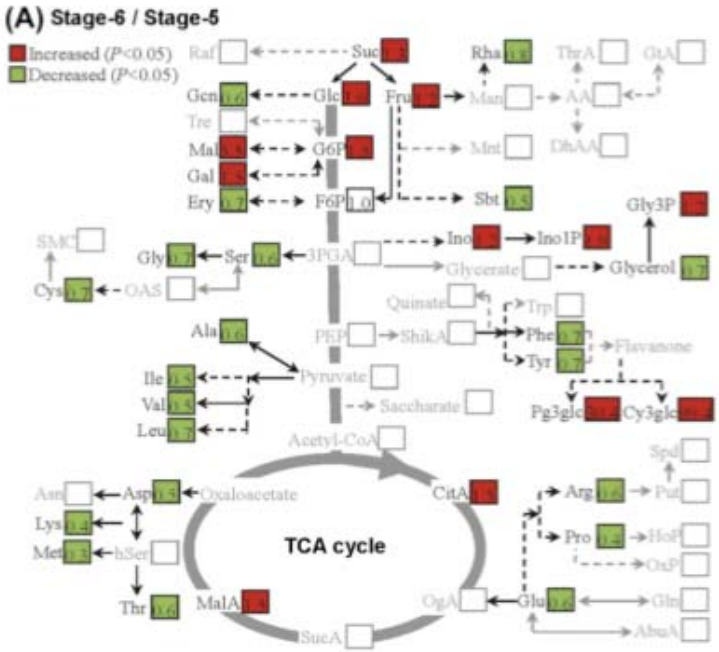


Fig. 6. PLS-DA score pl
strawberry fruit stages. T



Zhang et al,
JEB 2011

Tomate: effet d'un traitement pesticide (carbofuran) sur le métabolisme endogène

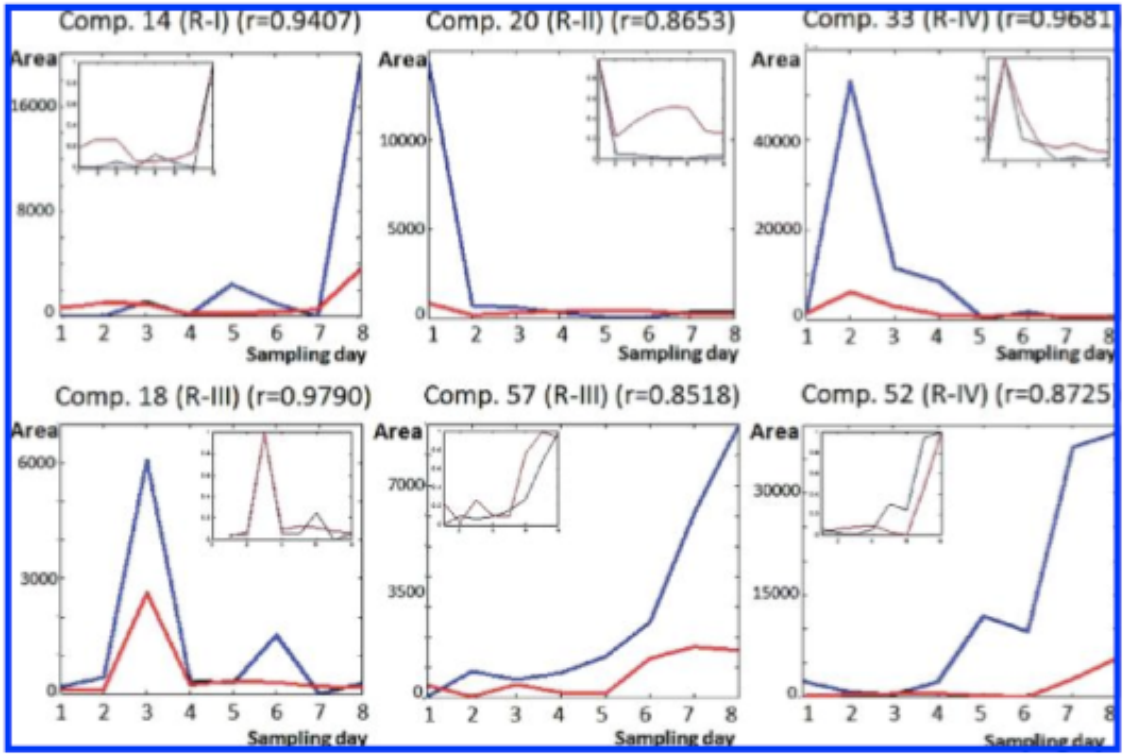


Figure 7. Evolutionary profiles of some components through the eight sampling days in treated (blue) and nontreated (red) samples, extracted from the four regions. The kinetic curves are similar, but the concentration level of the component in blank samples is lower than that in treated samples.

Sanchez-Perez
et al, Anal. Chem. 2009

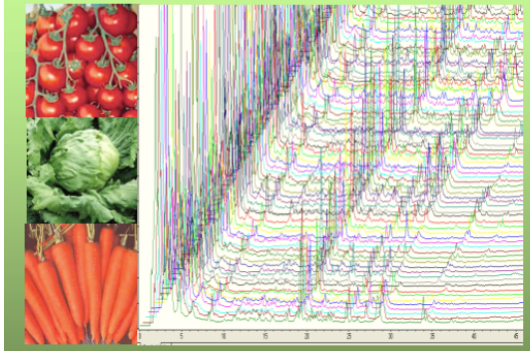
Comparaison qualité Bio / Conv
Rapport AFSSA 2001-03 (France) et
rapport FSA 2009 (UK): des différences modérées

Nutriment	Rapport AFSSA n= 310	Rapport FSA n= 162	
Matière sèche	Bio >	Bio > (+ 9,8 %)	
Magnésium	Bio >	Bio > (+ 7 %)	
Fer	Bio >	pas diff	
Zinc	pas diff	Bio > (+ 11,7 %)	
Ca, P, K, Cu, Mn	pas diff	pas diff	
Vitamine C	pas diff (> pomdete)	pas diff	Vit C > (+ 6%)
Acides Phénoliques/ Flavonoides	Bio >	Bio > (+ 13/38 %)	Phé > (+ 16%) Brandt, 2011
Acides gras PolyIns (produits animaux)	Bio >	Bio > (+ 10 %)	
Nitrates (légumes)	Bio < (- 50 %)	Bio N <	

Différentes méthodolgies utilisées pour des évaluations comparatives, basées sur des études sérieuses et en nombre suffisant (mais limité), donnent des conclusions assez fiables et concordantes,

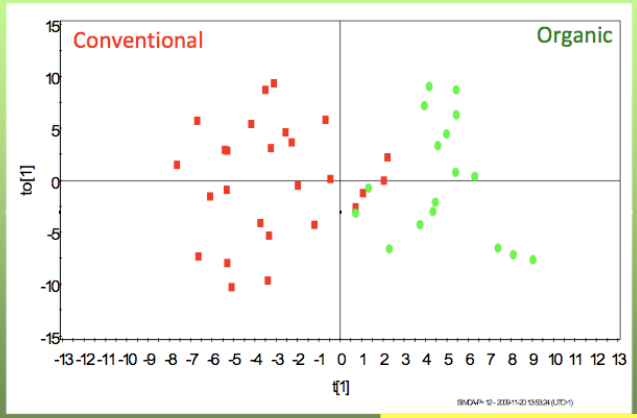
Comparaison Bio / Conv : Carrote et Tomate

Metabolomics



OPLS-DA on carrot samples

Model with 1 predictive LV and 1 orthogonal LV (Q2 = 0.38, CV-ANOVA = 10^{-3})

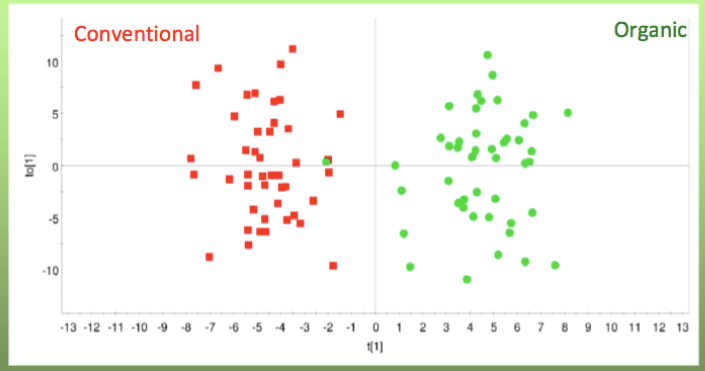


Organic carrots:
+ Sugars: Sucrose, sedoheptulose
+ AA: Ile/Leu, Aminoadipic acid
+ Malic acid

But chlorogenic acid in higher concentration in conventional samples

OPLS-DA on tomato samples

Model with 1 predictive LV and 3 orthogonal LVS (Q2 = 0.39, CV-ANOVA = 10^{-6})



Organic tomatoes:
+ Anti-oxidants : Chlorogenic acid, Coumaric and caffeic acid.
+ Natural pesticide: Tryptamine
+ AA: Gly, Ala, Leu, Tyr
+Alpha-tomatidine

Kelly S,. 2010

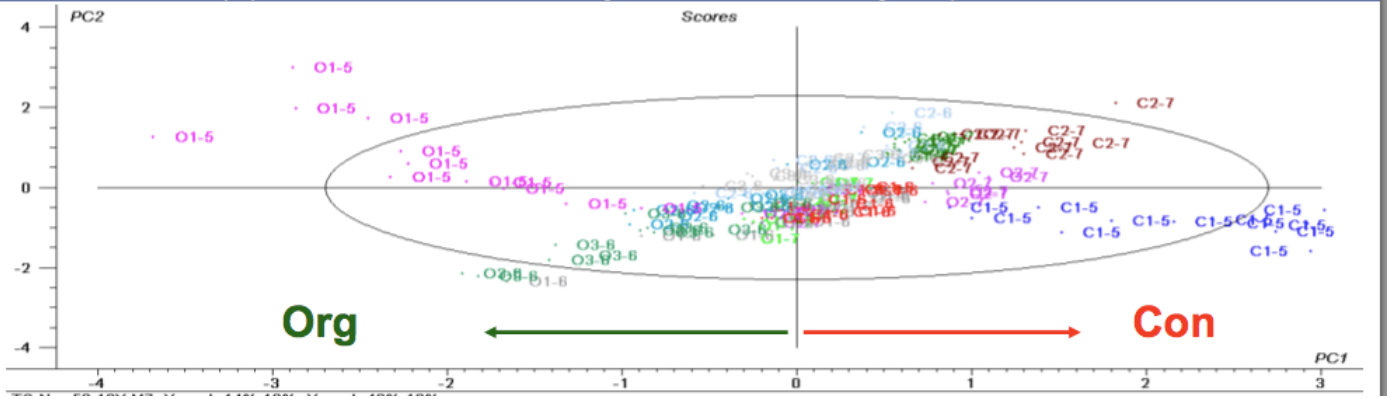
Carrote :
Comparaison Bio / Conv

JRC EUROPEAN COMMISSION

Results: PLS-DA on carrots

irmm Institute for Reference Materials and Measurements

JRC-IRMM, Geel (BE) on 30 November – 01 December 2009 – Organic Food Authentication: Challenge or Utopia? 20



JRC EUROPEAN COMMISSION

Results: PLS-DA models on carrots

irmm Institute for Reference Materials and Measurements

JRC-IRMM, Geel (BE) on 30 November – 01 December 2009 – Organic Food Authentication: Challenge or Utopia? 19

Independent PLS-DA models for each year

Year	r	PC #	RMSEC	RMSEP	Explained var. X	Explained var. Y	Pred. rate	Unknown test set 2005	2006	2007
2005	0.96	2	0.104	0.224	60 + 15	80 + 16	100%	-	67%	50%
2006	0.518	3	0.347	0.522	13 + 46	48 + 4	92%	88%	-	
	0.653	8	0.294	0.522	97	66	100%	100%		100%
2007	0.945	4	0.116	0.179	35 + 40	56 + 26	100%	50%	50%	-

- Models based on yearly data set have a high prediction rate
- Nonetheless in 2006, r is lower and RMSEP are higher
- It seems necessary to build a model each year

Maquet et al. 2010

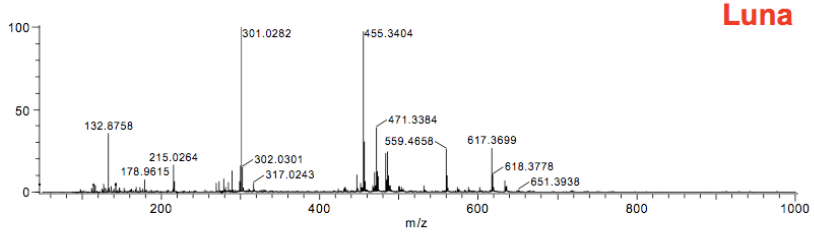
Pomme et pomme de terre : variétés et Comparaison Bio / Conv

DART–TOFMS [-] mass spectra

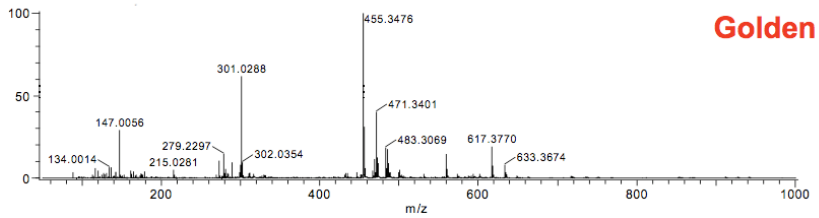
DART [-] : 350°C,PV 800V, MCP 2500V

25 markers

Luna



Golden delicious

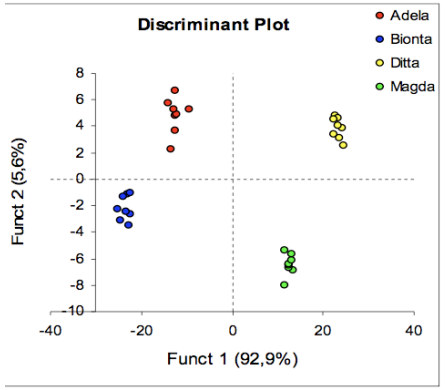


Identification of compounds - negativeion mode

Compound	mass	ion	Luna	Golden	Opál	Rozela	Topaz
pyruvic acid	87,0075	[M-H]-	+	+	+	+	+
Serine	104,0340	[M-H]-	+				
fumaric acid/ maleic acid	115,0024	[M-H]-	+	+		+	+
hexanoic acid	115,0751	[M-H]-	+	+	+	+	+
succinic acid	117,0180	[M-H]-	+	+	+	+	+
benzoic acid	121,0282	[M-H]-	+	+	+	+	+
5-oxoproline	128,0340	[M-H]-	+				+
malic acid	133,0129	[M-H]-	+	+	+	+	+
salicylic acid	137,0231	[M-H]-	+	+	+	+	+
2-oxoglutaric acid	145,01289	[M-H]-				+	
alditol/ xylitol/ Arabinitol	151,05983	[M-H]-	+				
Fru,Glc, inositol	179,0548	[M-H]-	+	+	+	+	+
citric acid	191,0185	[M-H]-				+	
linolei acid	279,2330	[M-H]-	+	+	+	+	+
olei acid	281,2486	[M-H]-	+	+	+	+	+
stearin acid	283,2644	[M-H]-	+	+	+	+	+
catechin, epicatechin	289,0721	[M-H]-	+	+	+	+	+
Quercetin	301,0356	[M-H]-	+	+	+	+	+
ursolic acid	455,3543	[M-H]-	+	+	+	+	+

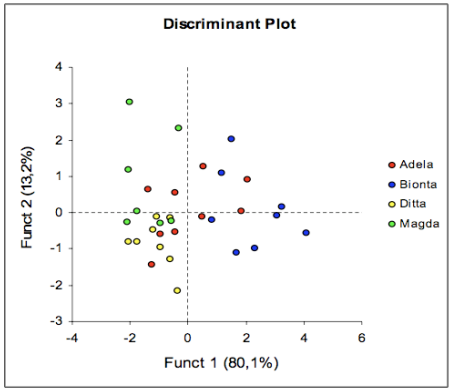
Potato study B

Grouping analysis, varieties - LDA



DART [–] mode

Prediction ability 62.5%



DART [+] mode

Prediction ability 12.5%

Potato study A - CONCLUSIONS

DART [–]

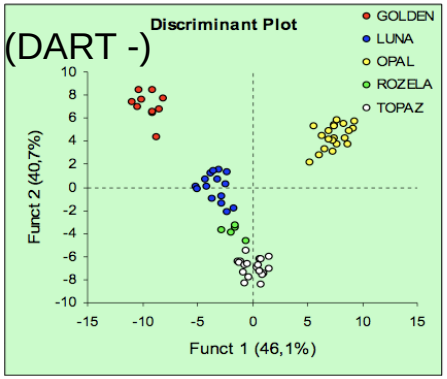
	Recognition ability	Prediction ability
Organic x conventional	100	65
Variety	100	45
Locality	100	80

DART [+]

	Recognition ability	Prediction ability
Organic x conventional	100	85
Variety	100	50
Locality	95	50

Hajslovà et al. 2010

Pommes : variétés et Comparaison Bio / Conv

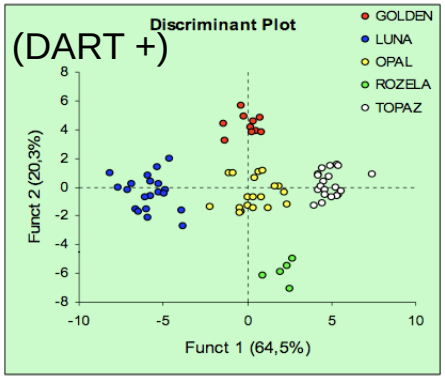


Differences between varieties

- **Recognition ability: 100%**
- **Prediction ability: 97.1%**

Note: **Prediction ability was obtained on the basis of leave-one-out cross validation (LOOCV)**

Software statistiXL 1.8



Differences between varieties

- **Recognition ability: 100%**
- **Prediction ability: 90.5%**

Note: **Prediction ability was**
of leave-one-out cross vali

CHEMOMETRIC ANALYSIS – LDA: Conventional vs organic

DART[-] – LDA: VARIETIES, 25 variables

Differences between farming

- **Recognition ability: 100%**
- **Prediction ability: 76.6%**

DART[+] – LDA: VARIETIES, 17 variables

Differences between farming

- **Recognition ability: 100%**
- **Prediction ability: 86.7%**

Hajslovà et al. 2010

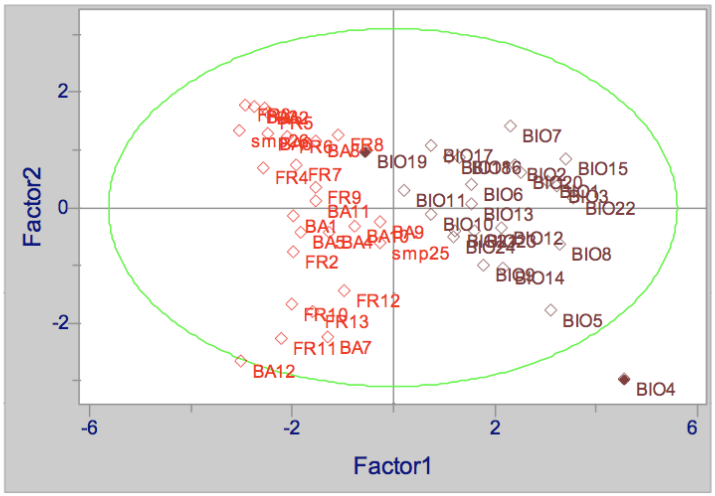
Œufs : Comparaison Bio / Conv

Organic vs. barn farming

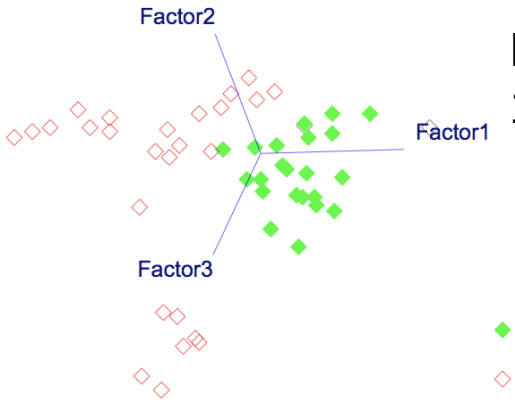
Balanced for location and for farm size

Acides gras

FA PLS-DA: autoscale, 2 component



PCA plot: carotenoids



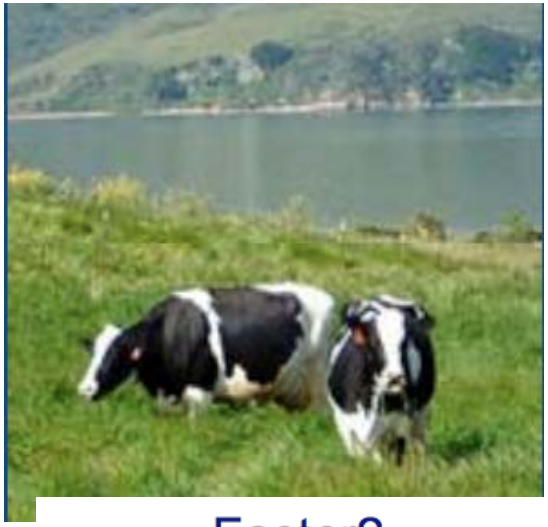
Prediction : 100% (24/24)

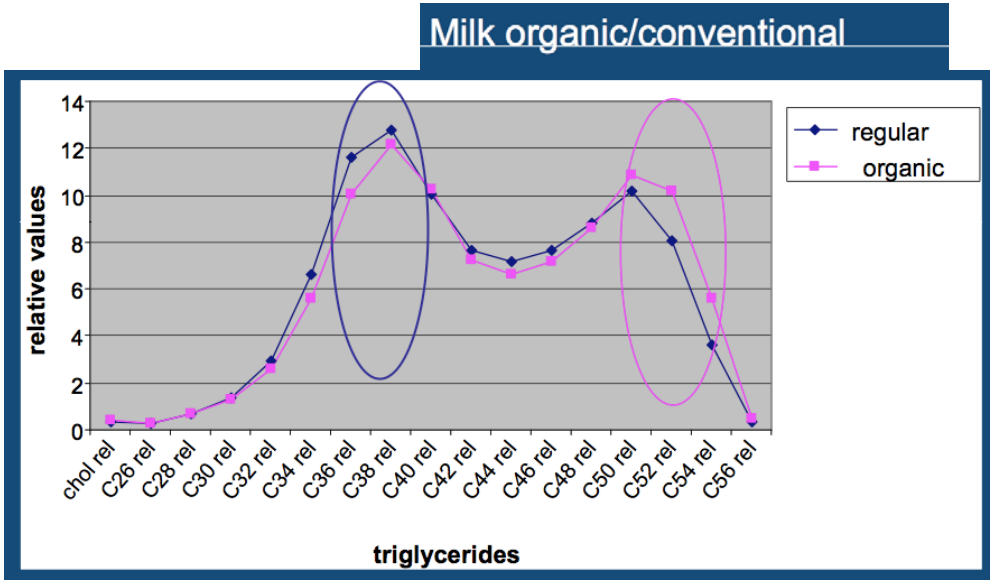
◆ = organic

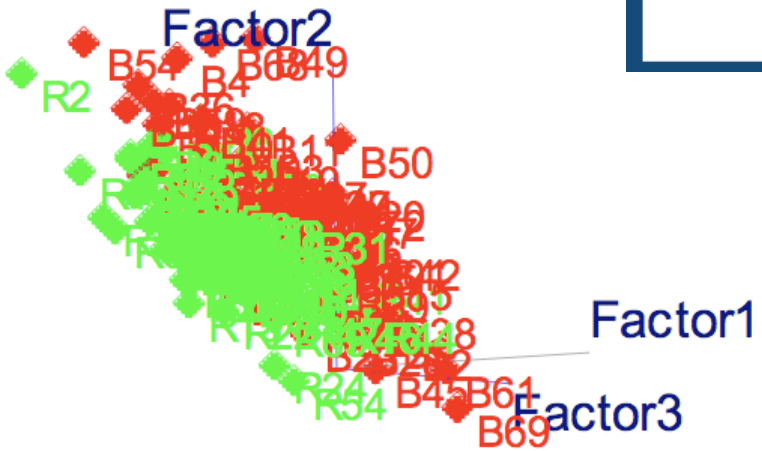
◇ = conventional

Van Ruth et al. 2010

Lait : Comparaison Bio / Conv



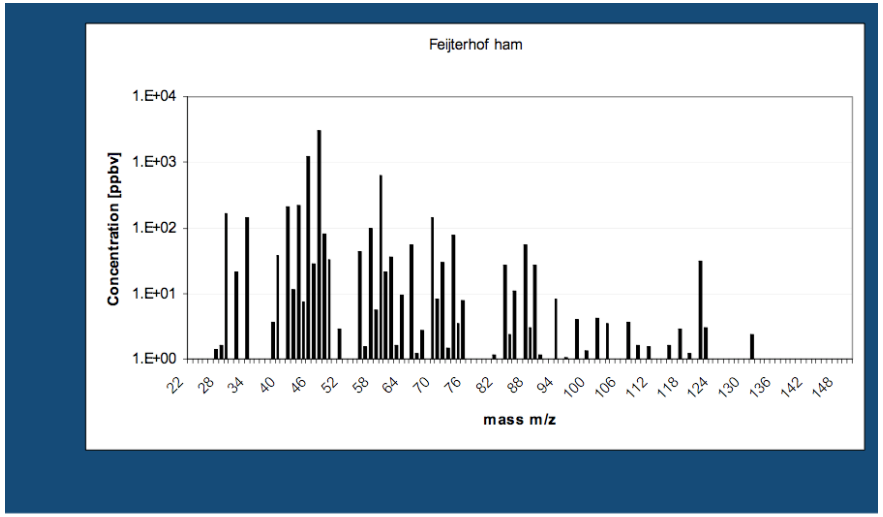


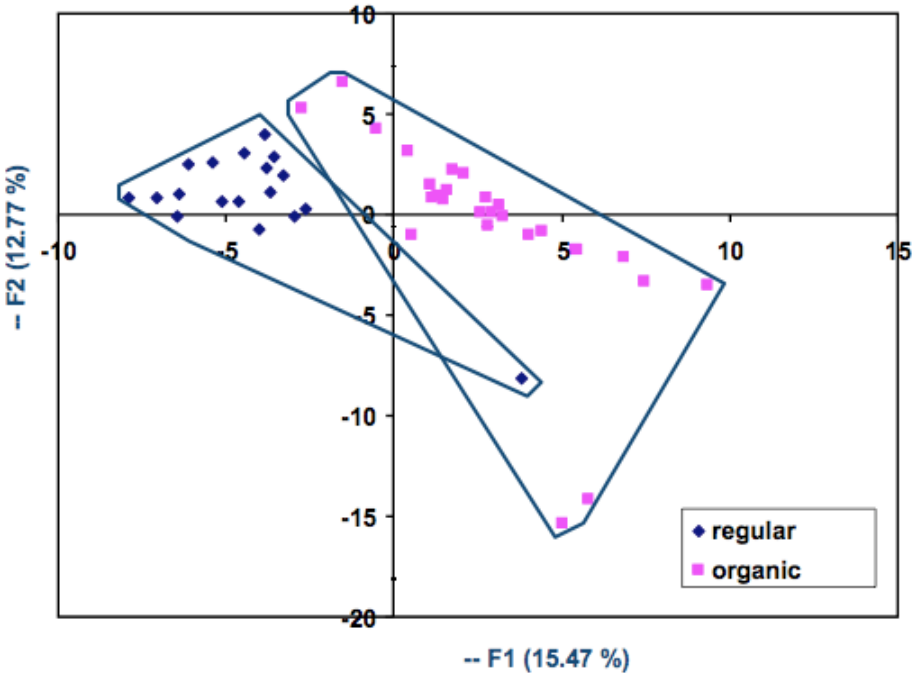


Classification :
Organic: 86 %
Convent. : 89 %

Van Ruth et al. 2010

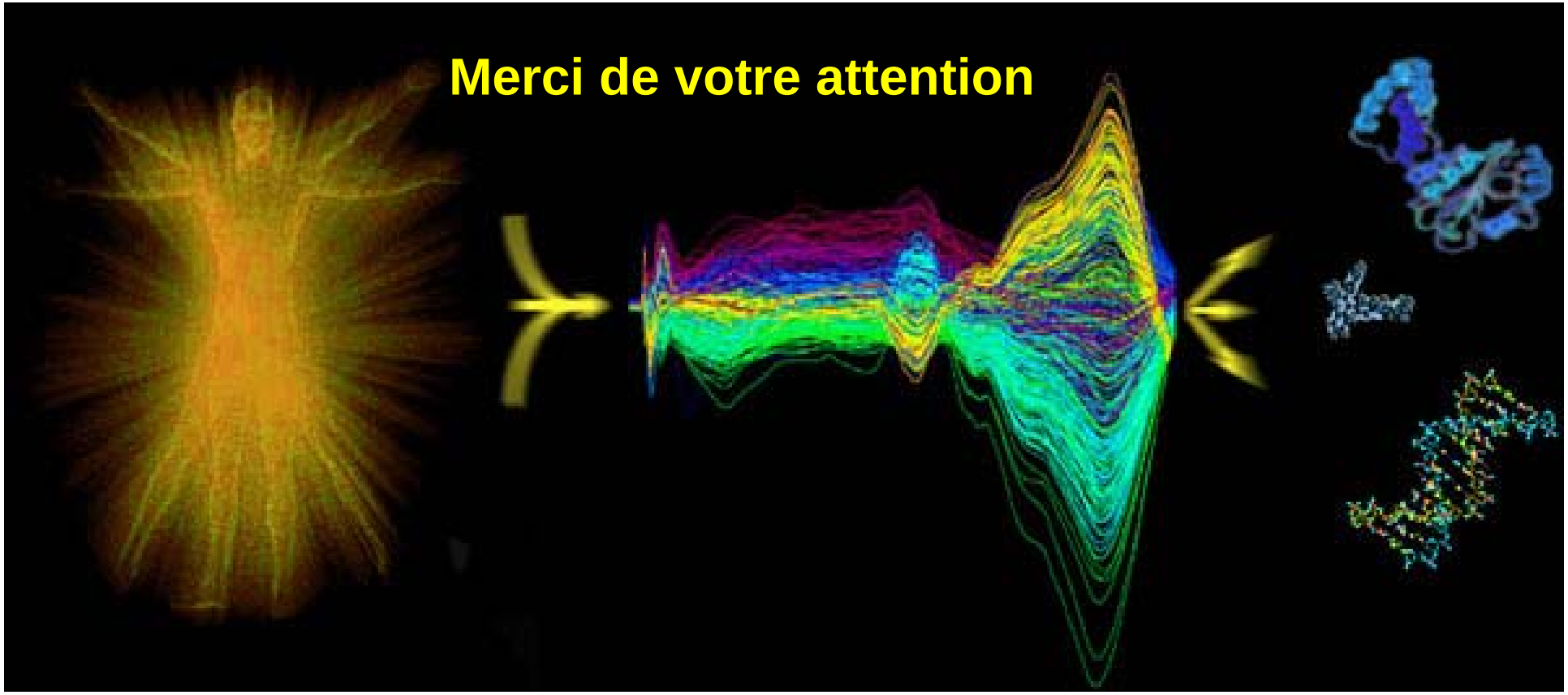
Jambon salé : Comparaison Bio / Conv





Classification :
Organic: 96 %
Convent. : 83 %

Van Ruth et al. 2010


Merci de votre attention

Métabolomique … …

… des données …

… à traiter

Manach et al., MNFR 2009

C. Manach *et al.*

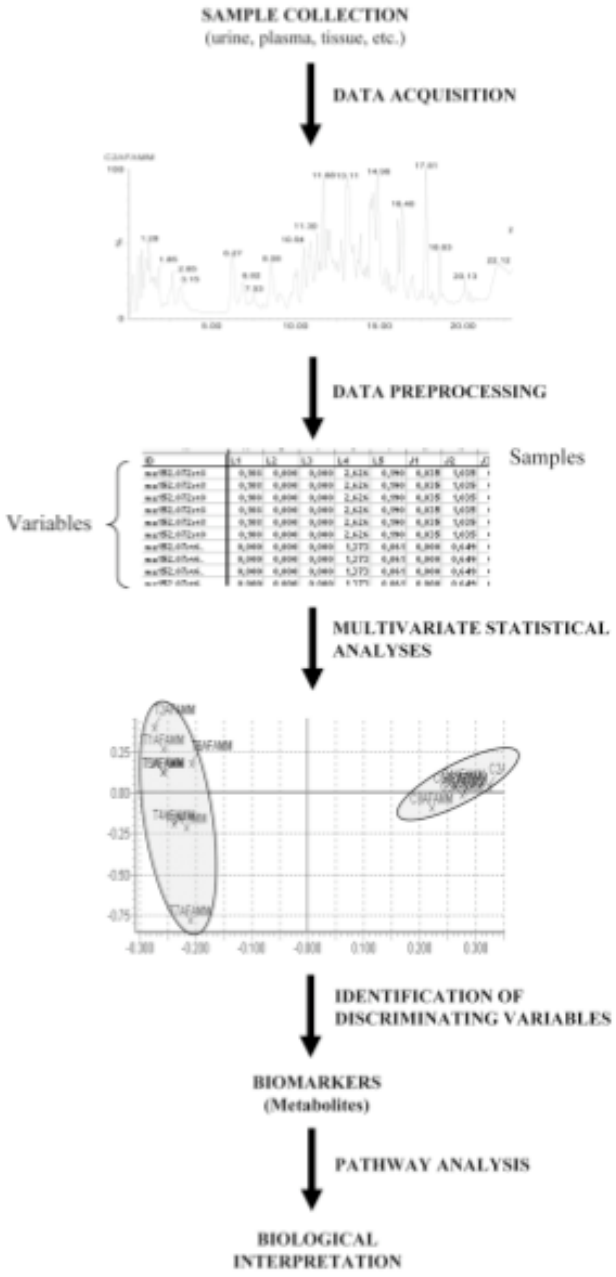


Figure 3. Flow process for metabolomic analyses.

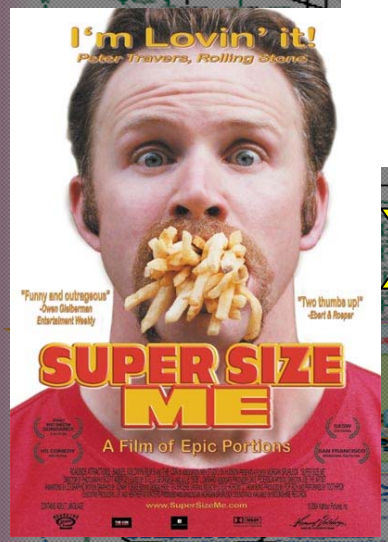
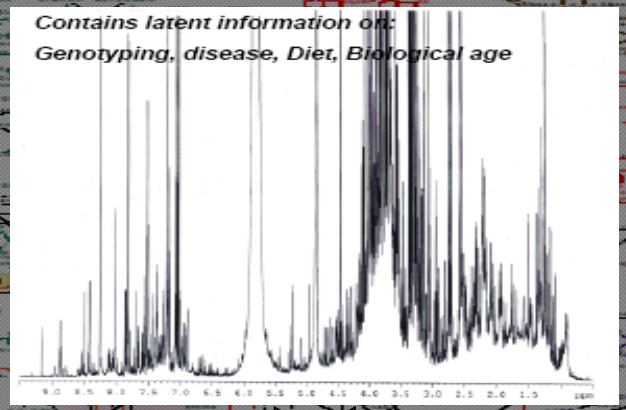
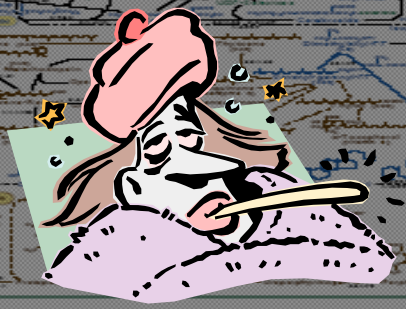


Plate-formes:
NMR
LC/MS
GC/MS

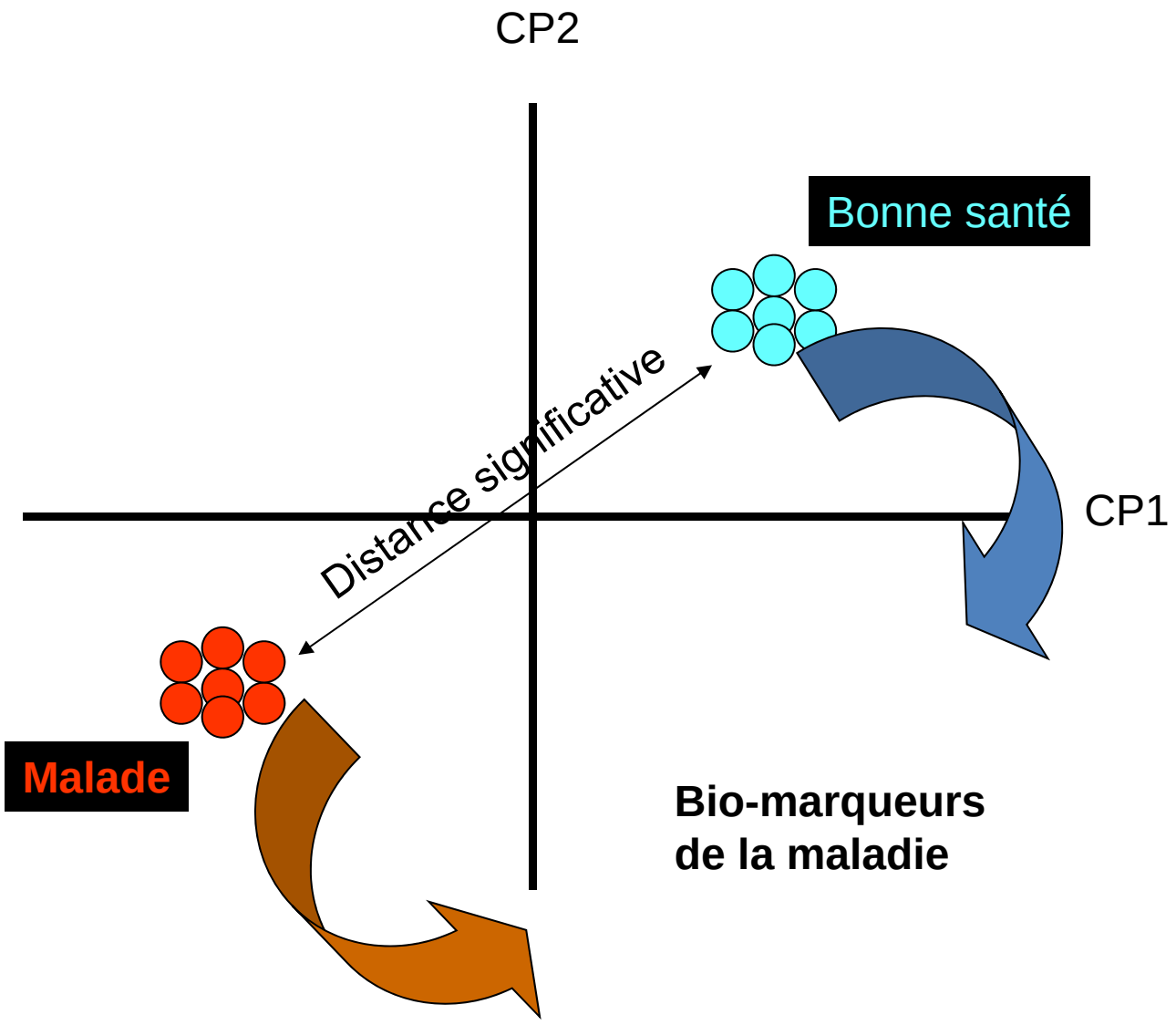


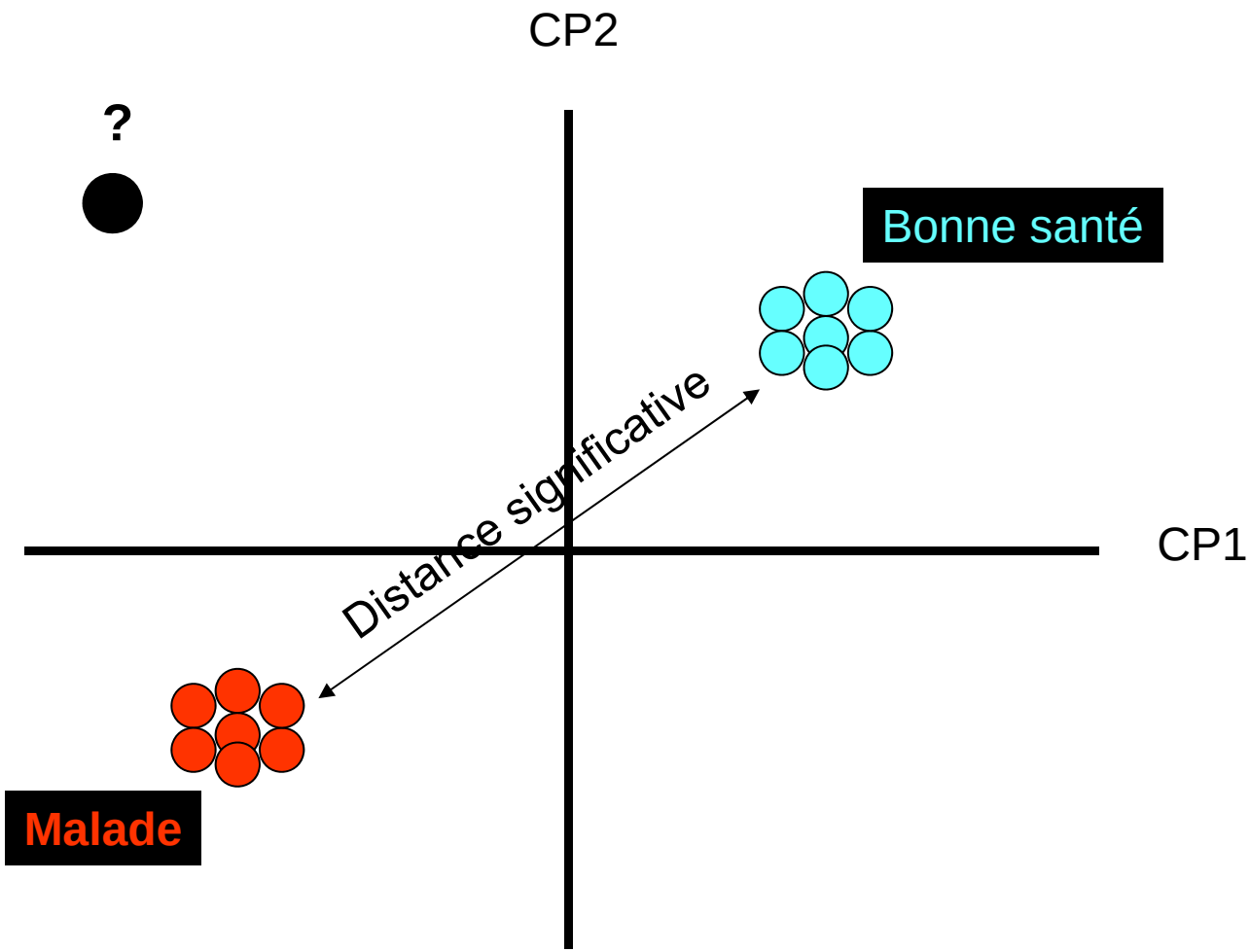
Data acquisition

MVA bioinformatic



Data mining

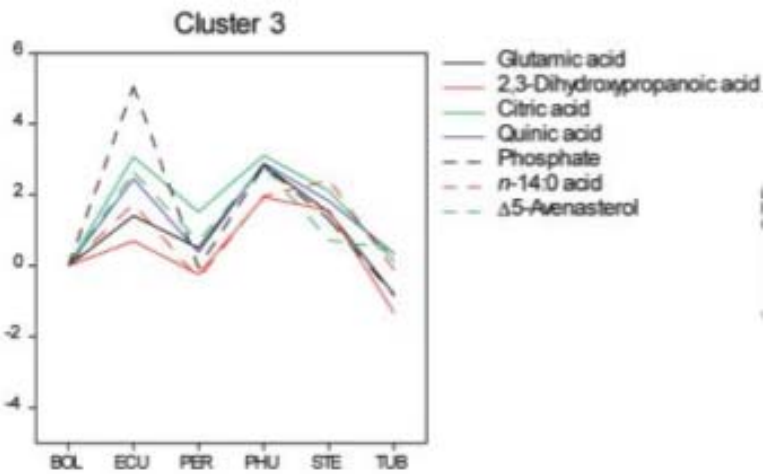

CP2
Bonne santé
Distance significative
CP1
Malade
Bio-marqueurs
de la maladie

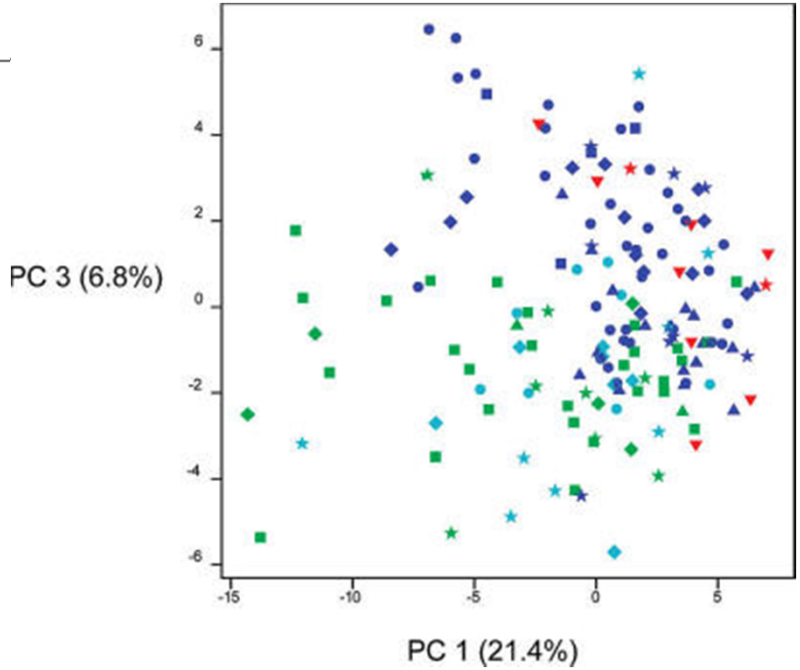

CP2
?
Bonne santé
Distance significative
CP1
Malade

Pommes de terre: effets des variétés

Table 1. List of Metabolites Used in the Analysis of Potato Tubers par GC-MS

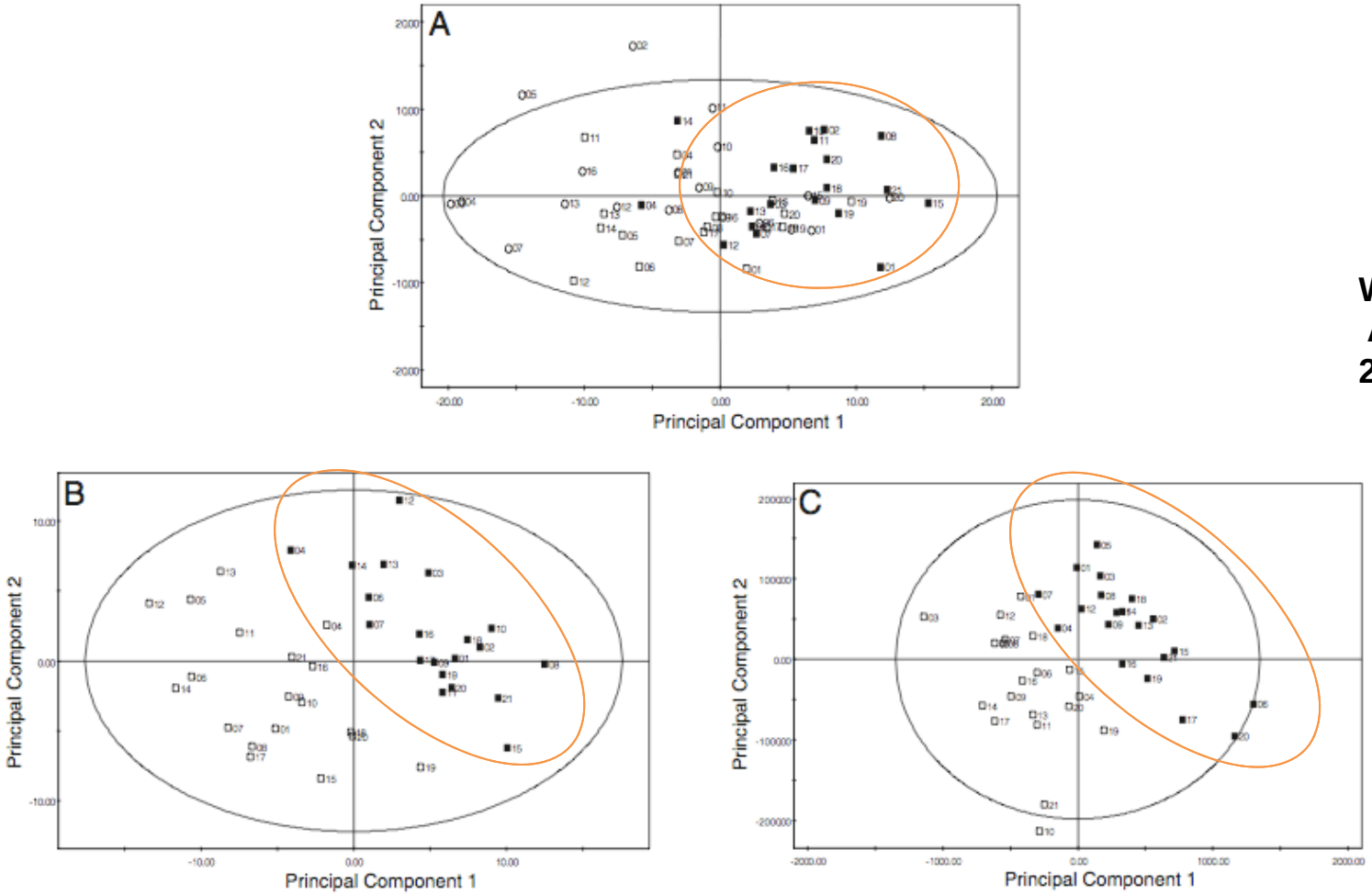
compound class	metabolite
amino acid	alanine, β-alanine, γ-aminobutyric acid, asparagine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine
organic acid	caffeic acid, citric acid, 2,3-dihydroxypropanoic acid, fumaric acid, 2-keto-1-gluconic acid,[a] malic acid, 2-piperidinecarboxylic acid (pipecolic acid), quinic acid, 2,3,4-trihydroxybutyric (threonic acid)
sugar[b]	carbohydrate1717, carbohydrate2363, fructose, glucaric or galactaric acid,[a] glucose, polysaccharide3062, polysaccharide3078, sucrose
sugar alcohol	inositol
other polar	*N*-ethyldiethanolamine,[a] phosphate.
unknown polar[b]	P1108, P1143, P1344, P1400, P1471, P1525, P1559, P1630, P1680, P1729, P1786
fatty acid[c]	*n*-14:0, *n*-15:0, *n*-16:0, *n*-17:0, *n*-18:0, *n*-20:0, *n*-21:0, *n*-22:0, *n*-23:0, *n*-24:0, *n*-25:0, *n*-26:0, *n*-28:0, *n*-29:0, n-30:0, *iso*-15:0, *iso*-17, 15:1, 16:1, 18:2(ω6), 18:3(ω3), 2-hydroxy-16:0, 2-hydroxy-24:0.
fatty alcohol[c]	*n*-21:0, *n*-22:0, *n*-23:0, *n*-24:0, *n*-26:0, *n*-27:0, *n*-28:0, *n*-29:0, *n*-30:0.
sterol	Δ5-avenasterol β-sitosterol, stigmasterol
other nonpolar	*n*-23:0 alkane, *cis*-ferulic acid, *trans*-ferulic acid, solanid-5-en-ol
unknown nonpolar[b]	N1785, N1868, N1914, N2268





Dobson et al, JAFC 2010

Homme : effets de régimes avec différents apports en Fruits-Légumes



Walsh et al., Am J Clin Nutr. 2007

FIGURE 1. A: Principal component analysis of nuclear magnetic resonance (NMR) urinary data. B: Partial least-squares-discriminant analysis (PLS-DA) of NMR urinary data. C: PLS-DA of mass spectrometry urinary data. ○, normal diet; ■, low-phytochemical diet; □, standard phytochemical diet. + 400 ml,g/j vs low-