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Minor Components as markers of olive oil authenticity and quality

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Overview

I. Importance of analysis and authentification of olive oil

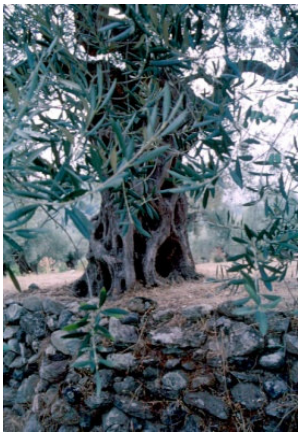
II. Methods for determination of
   1. Quality
   2. Genuineness

III. Authentication issues

IV. Olive oil components used in this study

V. Examples
   1. Harvest time
   2. Cultivar and geographic origin

VI. Conclusions



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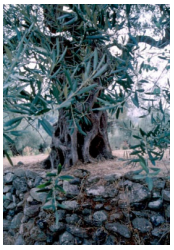
Importance of Analysis and Authentication of olive oils

- EVOO : mechanical extraction : unique composition and delicate aroma
- Lampante OO: needs refining
- Consumers preference: high quality + unchanged aroma and natural elements



➔ Higher price than other vegetable oils

➔ Danger of adulteration with cheaper ingredients

= economical fraud

= risk for consumers' health

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Importance of Analysis and Authentication of olive oils

Detect adulteration with cheaper ingredients

➔ Need for analysis and authentication methods

- Codex Alimentarius CA Commission Draft, 2003
- European Commission (EC) EC Reg No 2568/1991 and amendment 1989/2003
- International Olive Oil Council (IOOC) IOOC Trade Standards, 2003

= Official methods

➔ BUT sophisticated refining/adulteration

- Need for continual research

= new methods, not (yet) evaluated by standardizing bodies, but proposed by researchers

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Methods for olive oil quality verification

a) Methods included in the international Standards

- Olive oil sampling and laboratory sample preparation
- FFA
- PV
- Absorbances in the UV region
- Organoleptic assessment of VOO
- Volatile halogenated solvents in OO
- Metals
- **α-tocopherol** → **Addressed in this lecture**
- Moisture and volatile matter content
- Insoluble impurities in petroleum ether

b) Methods not included in the international Standards

- **Phenolic compounds** → **Addressed in this lecture**
- Volatile Compounds
- Partial Glycerides: MG, DG
- Accelerated oxidation tests
- Pigments (and derivatives)
- Contaminants

Methods for olive oil genuineness verification

Do the olive oil parameters correspond to the class it is said to be?
Genuineness: actually processing the alleged or apparent character

a) Methods included in the international Standards

- **FA composition**
- t-unsaturated FA
- FA in the 2-position of TAG
- ΔECN42 values
- **Sterol composition**
- Erythrodiol and uvaol
- Wax content
- Aliphatic alcohol content
- Stigmastadienes
- Spectrophotometric analysis in the UV region

b) Methods not included in the international Standards

- **Triacylglycerols**
- Alcoholic fraction
- Other abosorptions in the visible and UV spectra
- Hydrocarbons

Authentication issues

~ Traceability
- Adulteration with other vegetable oils
- Olive Variety
- Geographical Origin

- Adulteration
- Current Problems
  - Addition of hazelnut oil
  - Olive oil subjected to forbidden deodorization in mild conditions
  - mixtures of virgin olive oil with olive oil obtained by second centrifugation of the olive pastes (Remolido)
- Varietal Characterization
- Characterization of virgin olive oil by geographical origin

Influencing factors

olive variety
geographical location
ripeness of olives
agricultural practices
storage conditions of olives
processing
storage conditions of oils
...

Oil composition

Fatty Acids composition
Triacylglycerol composition
Tocopherols (α-tocopherol)
Phytosterols
Polyphenols
...

Quality

Authenticity

Fatty acids composition

FA composition and Olive oil genuineness (evoo)

- C18:1 : 55-83 %
  typical for olive oil : quality (stability, health) + authenticity
- C18:3 ≤ 1 % (highest in Moroccan genuine oo)
- Minor FA prominent in seed oils:
  - C20:1 ≤ 0.4 % <> adulteration with SFO, RSO
  - C22:0 ≤ 0.2 % <> adulteration with SFO, RSO
  - C22:1 ND <> adulteration with RSO
  - C24:0 ND <> adulteration with peanut oil
- ! Limits not useful if adulteration with ≤ 5% of seed oil
- FA composition similar for hazelnut oil, high oleic sunflower oil
  ➔other analyses needed
- Analysis: GC of FAME

Triacylglycerol composition

- TAG in authenticity assessment**:** evidence mixing with
  - Re-esterified (olive) oils
  - Hazelnut oil
- LLL cfr. supra: useful BUT addition canola oil till 7.5% not detected
- ΔECN42 value (~Δ LLL)
  - Desterolized seed oils on the market with similar FA composition as olive oil

  ➔ adulteration **not** discernible from FAME analysis
  - Falsification: seed oils: high content in ECN42 TAG (LLL)
  - Detection of falsification: **Δ**ECN42 = | LLLreal - LLLtheor | ≤ **0.2 (EVOO)**

    LLLreal = LLL content from HPLC analysis of TAG

    LLLtheor= LLL content, theoretical, calculated from FAME
- Analysis :
  - Oil dilution;
  - RP-HPLC + RI or ELSD
  - GC (phenyl-methyl-silicone phase)

Tocopherols (mainly α-tocopherol)

- In oil: Antioxidant
- Human health: vitamin E

- content: 100-300 ppm (good quality olive oils)
- composition: 90% α–tocopherol
- ↓↓ during refining ➔ Added to refined (seed) oils to ↑ ox.stab. (allowed)

- Analysis
  - Oil dilution in hexane
  - Separation/Quantification: normal phase HPLC + fluorescence detection

Phytosterols

- in plant: part of cell membranes; very characteristic for each botanical species
- Human health: Antihypercholesterolemic
- Content in olive oil: 1000-2000 mg/kg
  - Refining ➔ ↓ sterols (dehydration) ➔ formation of steradienes
  - Adulteration with seed oils ➔ content > 2000 mg/kg
- Composition: characteristic content and profile in olive oils
  - desmethylsterols, 4-methylsterols, 4-dimethylsterols
  - In this study:
    - β-sitosterol
    - Campesterol
    - Stigmasterol
    - 24-methylene cycloartanol
    - cycloartenol
    - citrostadienol
- Analysis
  - Saponification
  - Extraction unsaponifiables
  - Gas chromatograpy of unsaps

Phenolic compounds

- In oil; human health: antioxidant
- Content: 50-1000 mg/kg (usual: 100-300)
- Composition: Characteristic content and profile in olive oils
  - Simple phenols, phenolic acids, lignans, flavonoids
  - In this study:
    - **Hydroxytyrosol**
    - **Tyrosol**
    - Vanillic acid
    - p-coumaric acid
    - Vanillin
    - Lutein
    - Apigenin
- Analysis
  - **SPE** Extraction + clean-up
  - **Total phenols** content: Folin-Ciocalteu colorimetric method
  - **Individual phenols**: Separation on HPLC: RP-HPLC-UV (280 nm) + MS

Examples

Oils from European and non-European countries

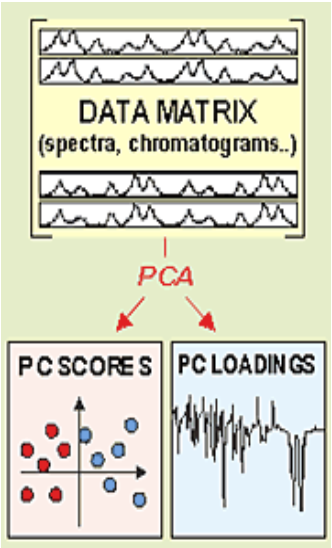
Analysis of characteristic composition

Principal Components analysis of results

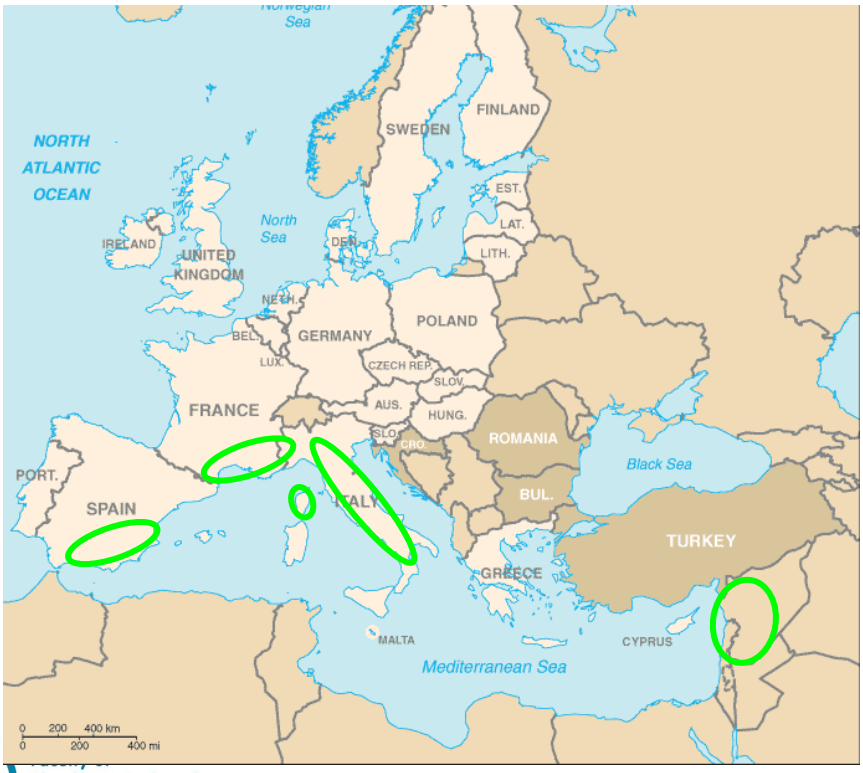
Clustering of similar olive oils

Cultivar

Origin



PCA analysis on olive oils



http://www.drakecorp.com/s_distribution/ee-map.gif

Examples: Oils from

- Spain
- South-France
- Corsica
- Italy
- Syria

Analyses

- **Fatty Acids**
- **Triacylglycerols**
- **Tocopherols (α-tocopherol)**
- **Phytosterols**
- **Polyphenols**

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PCA analysis on olive oils

Geographical Origin	Cultivars
Spain	Picual, Verdial, Cornicabra
South-France	Aglandou, Cailletier, Tanche, PDO Aix en Provence & Vallée des Baux
Corsica	Ghjermana, Zinzala, Sabina
Italy	Many different cultivars, each in 1 repetition: Pisciottana, Tonda Iblea, Ottobratica, Gentile di Chieti, Carolea, Ogliarola Leccese, Cellina di Nardo, Nocellara del Belice, Coratina, Taggiasca, Bosana, Dritta, Tonda di Cagliari, Cima di Mola, Peranzana, Sinopolese
Syria	Zayti, Sourani, Nibali, Commercial

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http://www.drakecorp.com/s_distribution/ee-map.gif

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1. FAME and TAG

Oils of 2 countries:

•Italian I

Higher PUFA

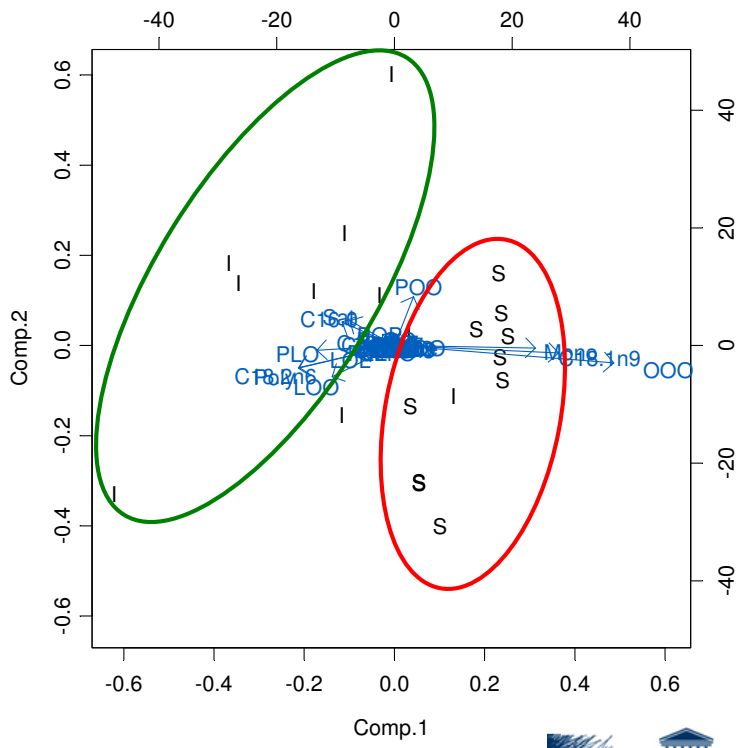
•Spanish S

Higher MUFA

In this case:

Separation based on country

No cultivars separation



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1. FAME and TAG

Oils of 3 countries:

•Italian I

Higher PUFA

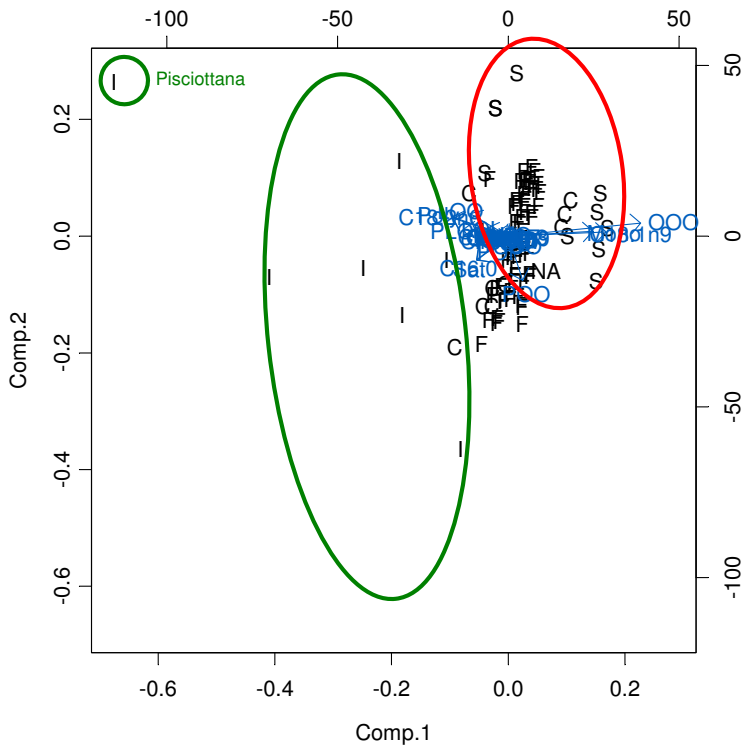
•Spanish S

Higher MUFA

•Corsican C

•French F

Both: intermediate



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2. FAME and TAG + sterols + tocols

Oils of 2 countries:

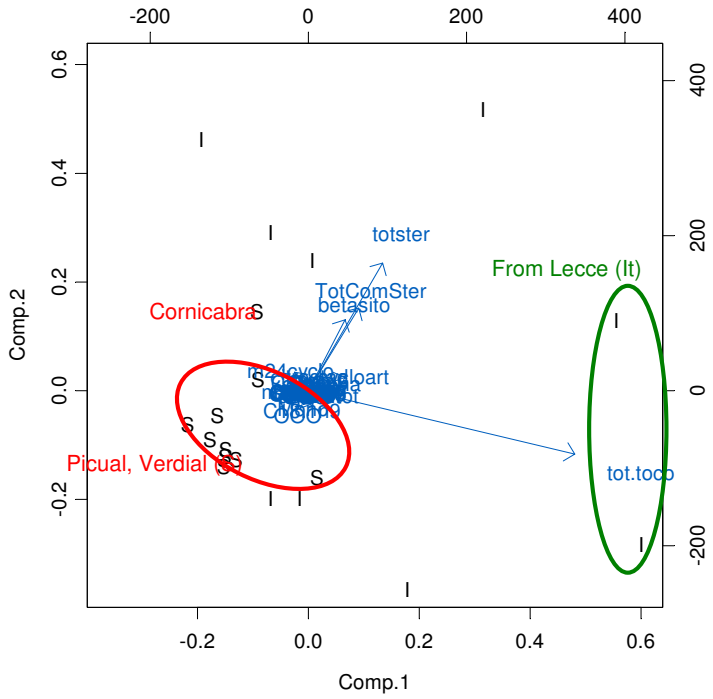
•Italian I

All different cultivars: scattered;

Slight distinction for oils from Lecce

•Spanish S

Picual & Verdial: separated from Cornicabra (mainly: sterols composition)



2. FAME and TAG + sterols + tocols

Oils of 3 countries:

•Italian I

Many different cultivars (and regions) ➔ scattered

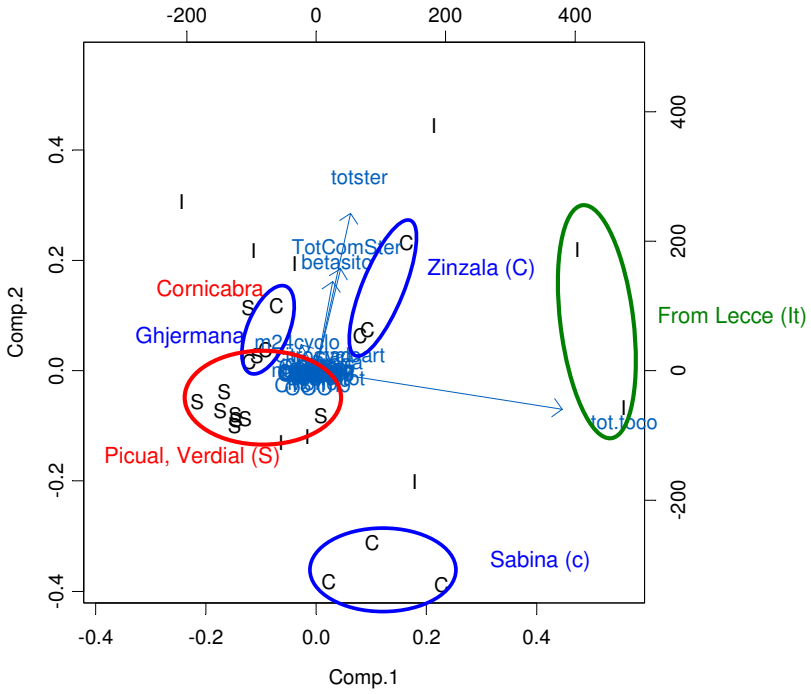
•Spanish S

Picual, Verdial : close

Cornicabra: further

•Corsican C

Ghjermana, Zinzala, Sabina (poorest in sterols) cultivars: separated

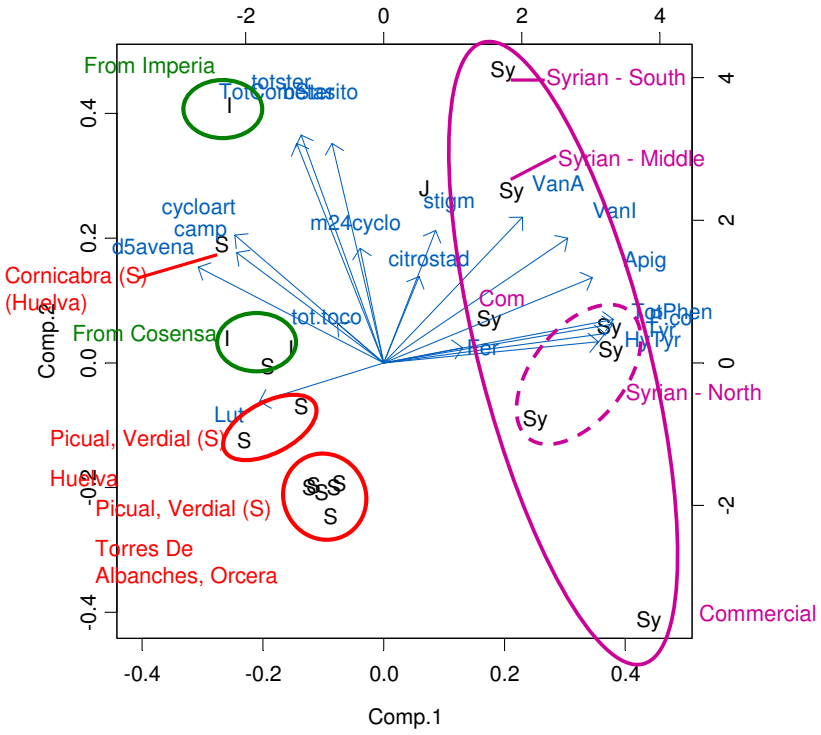


3. Sterols + tocols + phenols

Example:

Oils of 3 countries:

- Italian I
- Spanish S
- Syrian Sy
- (Jordan: J)



4. FAME, TAG + sterols + tocols + phenols

Example: Spanish extra virgin olive oils

Cultivar	Region (Spain)	Subregion
Cornicabra	Castilla	Toledo
Verdial	Andalucia	Huelva, Villarrasa
Picual		
Verdial		
Picual		
Verdial		Huelva, Bartolina: 50 km from Villarasa
Picual		Jaen, Puerta de Segura
Picual		Jaen, Torres de Albanchez
Picual		Jaen, Orcera
Picual		

Oils: Kindly provided by: Prof. A. Martin & P. Hernandez, CSIC, Spain

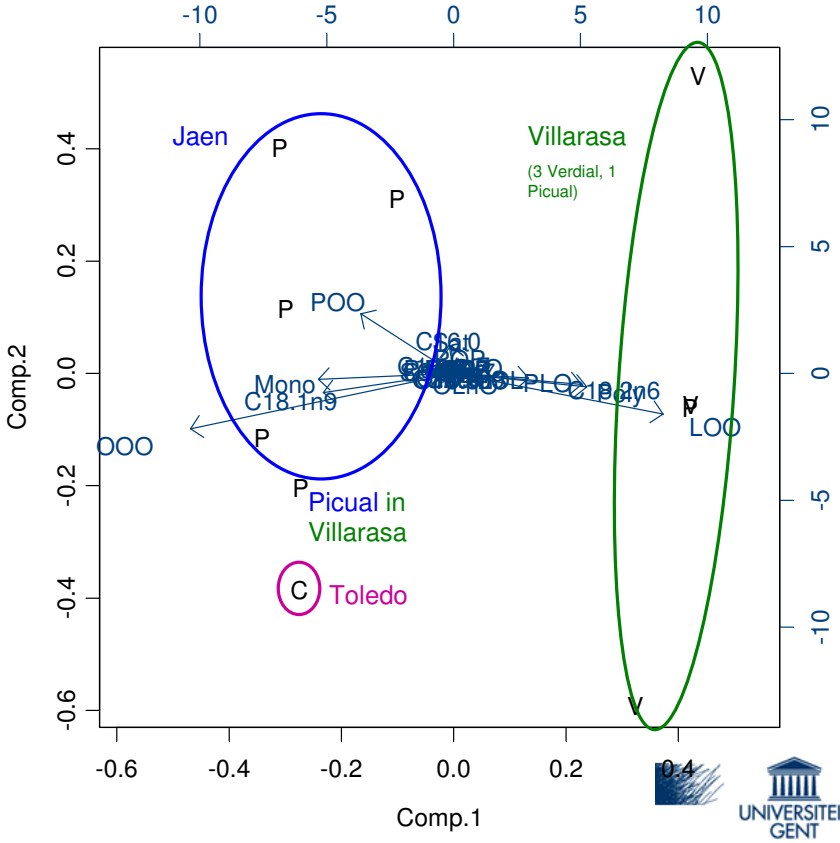
4. FAME, TAG + sterols + tocols + phenols

FAME + TAG

P: Picual
C: Cornicabra
V: Verdial

Region

Cultivar



4. FAME, TAG + sterols + tocols + phenols

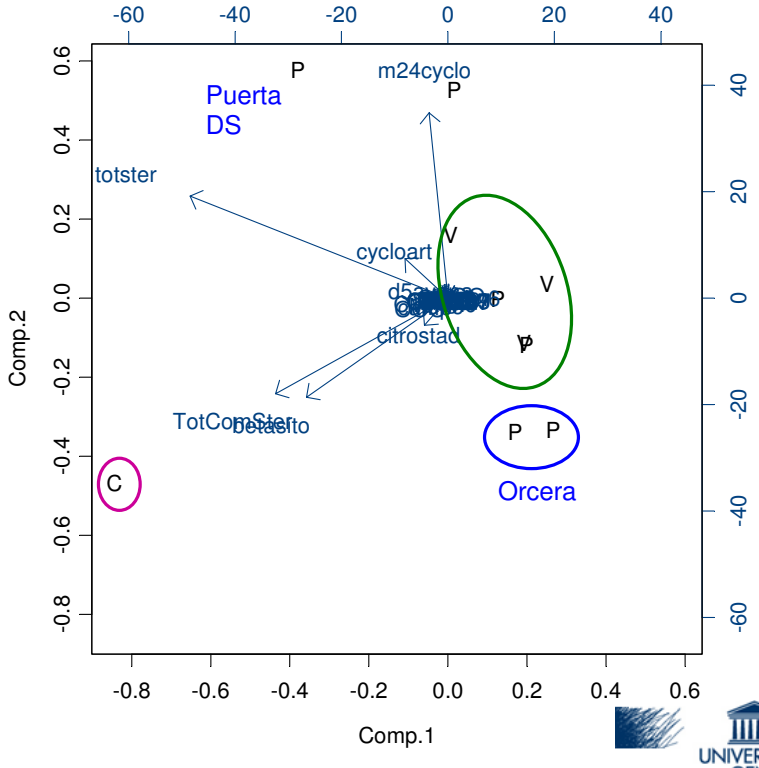
FAME + TAG + sterols

P: Picual
C: Cornicabra
V: Verdial

Region

Subregion

Cultivar



4. FAME, TAG + sterols + tocols + phenols

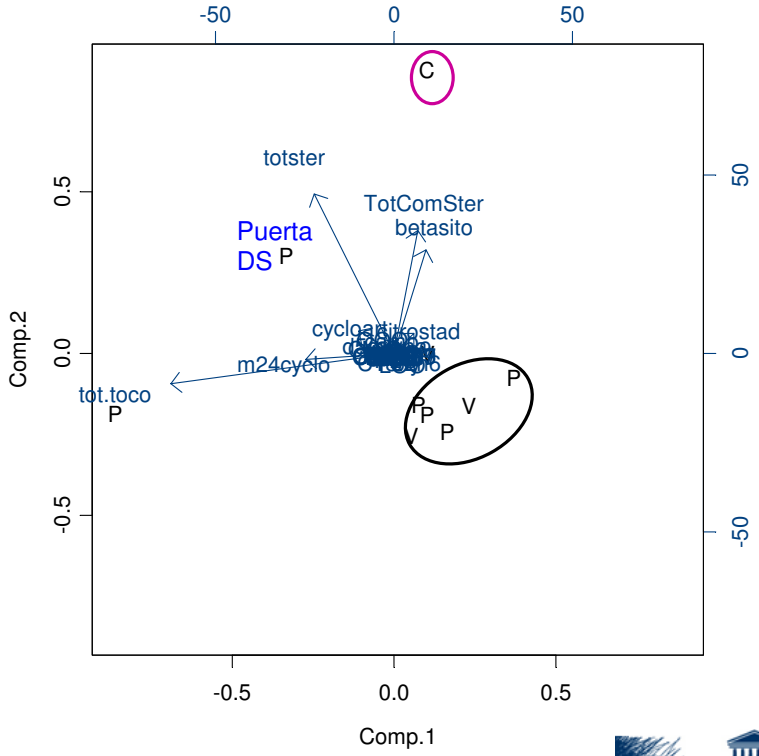
FAME + TAG + sterols + toco

P: Picual
C: Cornicabra
V: Verdial

Region

Subregion

Cultivar



4. FAME, TAG + sterols + toco + phenols

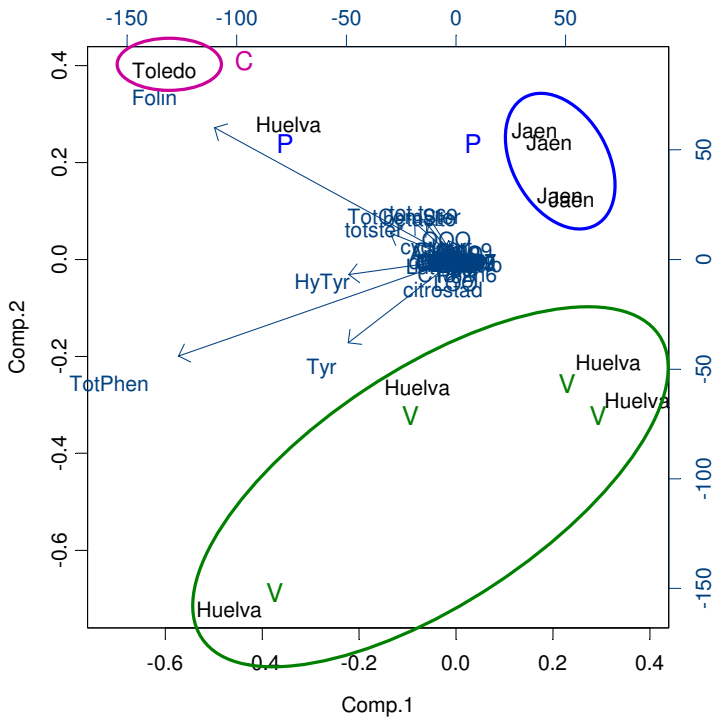
FAME + TAG + sterols + toco + phenols

P: Picual
C: Cornicabra
V: Verdial

Region

Subregion

Cultivar



Conclusions

- Extra virgin olive oil: high quality, high price ➔ adulteration
  - Lower quality olive oils — QUALITY
  - Other vegetable oils — AUTHENTICITY
- Detection: chemical analyses: FA, TAG, minor components
  - ➔ Clustering of oils in groups ~ Cultivar ; Region ; Subregion
  - ➔ FA + TAG ➔ main groups
  - ➔ + minor compounds: Sterols, tocopherols, polyphenols
    - ➔ more detailed separation: Region, subregion, cultivar, agricultural practices
- Improving models
  - combination of various components
  - Extension number of samples
  - ➔ New tools in determination EVOO quality and authenticity

Thank you

for your attention!

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