Olive oil phenolics and health: Innovation tools

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Some of the authors of this publication are also working on these related projects:

ARISTOIL - INTERREG MED PROGRAMME (2014 - 2020) View project
Dietary Bio-actives and Metabolic Reponses View project
HOW HEALTHY IS YOUR OLIVE OIL?
INNOVATIVE TOOLS FOR THE OLIVE OIL INDUSTRY

Prokopios Magiatis & Eleni Melliou
Pharmacognosy

(pharmakon+gnosis) is the science of identifying natural sources of drugs either for therapy or for protection of health.

In my eyes olive fruits and consequently olive oil is a herbal drug that is so commonly consumed in the Mediterranean diet that we usually forget its crucial role in health.

Several examples of very common foods have been officially recognized as sources of herbal medicinal products by the European Medicines Agency (Tea, coffee, turmeric, garlic).
Food or drug?

- Olive oil is not a simple food or a simple source of lipids.
- It contains unique minor chemical compounds with delicate structures that can enter in the human body through the usual diet and have an impact on health.
- The only known edible source of these specific chemicals is the olive fruit and the olive oil.
- Olive oil is in the intermediate between food and drug providing an excellent example of Hippocratic medicine.
Olive- olive oil

Dioscorides and after him all the ancient doctors insist that the best health effects come from the fresh olive oil from unripe olives or from specific varieties **but not from all olive oils**

Numerous applications are reported including headache, toothache

Obvious indications of antinflammatory activity

*Dioscorides Pedanius Med., De materia medica.* Ἐλαιον πρός τὴν ἐν ύγιείᾳ χρήσιν ἀριστον τὸ ωμοτριβές, δι καὶ ὁμφάκινον καλούσι.
Oleocanthal (decarboxymethyl ligstroside aglycone) possesses antiinflammatory activity similar to Ibuprofen.
- COX1 and COX2 inhibitor
- Inhibition of tau fibrillization protection from Alzheimer’s disease in vivo
- Treatment of rheumatoid arthritis
- Against tumor promotion

Chronic exposure to low doses of antiinflammatory agents like oleocanthal offers protection against cardiovascular diseases and aging.

Oleocanthal is responsible for the pungency of fresh olive oil coming from unripe olives (irritation of oropharyngeal region).

Oleacein

Oleacein has a similar structure with oleocanthal.

It is a derivative of hydroxytyrosol and it is the most powerful antioxidant constituent of olive oil and also an inhibitor of LOX
The new EU regulation

- 5 mg of hydroxytyrosol and derivatives (oleuropein complex and tyrosol) per day offer protection against LDL oxidation.

- Oleocanthal and oleacein are the two most abundant forms of conjugated hydroxytyrosol and tyrosol in most olive oils, together with oleuropein aglycon and ligstroside aglycon.

- Our target was to develop a reliable method to measure all the compounds mentioned in the regulation in one experiment and provide the necessary data for the health claims.
The problem

- All the tyrosol and hydroxytyrosol derivatives found in olive oil present several technical difficulties in their chemical analysis and the existing data are not reliable.
- They are sensitive to polar solvents (water or methanol) or silica based stationery phases used for chromatographic quantitation (HPLC-UV or LCMS).
  - We have proven that they react with water or methanol or in any buffer with pH>9 and their analysis in aqueous media is problematic.
- The existing chromatographic methods are not reliable. Numerous publications have used the problematic chromatographic method and should be revised.
The answer

To avoid chromatography, we developed an innovative tool for the measurement of the oleocanthal and oleacein levels by quantitative $^1$H-NMR in CDCl$_3$ at 400, 600 or 800 MHz in order to identify possible differences between extra virgin olive oils and to classify them according to their potential health effects.

In JAFC 2012 and JAFC 2014 we have shown that using qNMR the measurement can be achieved in easily prepared olive oil extracts in less than 15 min per sample.

TYPICAL EXAMPLE OF $^1$H-NMR OF THE ALDEHYDIC PROTONS REGION OF OLIVE OIL EXTRACT AT 600 MHZ
Comparison between extra virgin olive oil total extracts

High quality

Low quality
Obvious differences among varieties

Each variety presents a unique profile in the 9.1-9.8 ppm region in the H-NMR spectrum. IS: internal standard. The signals for quantitation of oleocanthal and oleacein are noted as 1 and 2 respectively. Peaks 3 and 4 correspond to 3,4-DHPEA-EA and p-HPEA-EA respectively.

A: Koroneiki variety (with typical oleocanthal/oleacein ratio), B: Arbequina variety (with inversed oleocanthal/oleacein ratio), C: Mission variety, D: Megaritiki variety
Impact of maturity during harvest

Same olive grove, same variety, same olive mill
New index

A series of new indexes for the characterization of extra virgin olive oils has been proposed:

- D1 = oleocanthal + oleacein level
- D2 = oleocanthal/oleacein ratio
- D3 = sum of four secoiridoids
- D4 = oleacein + oleuropein aglycon (HT derivatives)

D to remind UC Davis and the people there that encouraged us to complete this work.
Oleocanthal

- Following the ancient guidelines we have recently identified olive oil varieties with >1000 mg/Kg oleocanthal
- NMR screening of >1000 different olive oils (variety+geographic origin+harvest time+processing)
- 25 gr olive oil per day can offer >15 mg oleocanthal
The selective pulse method

Nonselective $^1$H (blue) and 11-8 PPM SELDPFGPE spectrum (red) of EVOO in CDCl$_3$. 
225 mg olive oil (ca 250 μL) were mixed with 500 μL CDCl₃ containing Syringaldehyde as internal standard (50 μg/mL) and transferred to tube.

Data from 16 scans were collected in the 9-11 ppm region. The total experiment lasted 3 min.

The experiments were performed using a Bruker 600 MHz with cryoprobe at UC Davis.
Comparison between excitation pulse (up) and normal $^1$H-NMR of extracted oil (down)

- hexenal
- hexanal
- oleacein
- oleocanthal
Advantages

The selective excitation pulse method presents significant advantages compared to the extraction method (no work up, less time).

Currently it is the fastest and simplest method available for screening if an oil can have a health claim.
We developed a cheap and simple colorimetric test with which anybody can measure the levels of oleocanthal and oleacein and to evaluate the quality of olive oil.

A small quantity of olive oil is mixed with a specific reagent and after some time later a color appears. The intensity of the color (from light yellow to green) is selectively related with the concentration of oleocanthal and oleacein.

The test is protected with a Greek patent and is already available for demonstration and soon will be commercially available.

www.aristoleo.com
Oleokoronal

- New compounds isolated for the first time in olive oil and in nature
- The olive oil after thousands of years of human use it continues to surprise us with its secrets

Oleokoronal: 1\textsuperscript{st} detection in olive oil of koroneiki variety

Oleomissional
Impact of malaxation time and temperature

34 °C 70 min

34 °C 45 min

34 °C 30 min

Same olives, same day, same olive mill. The phenolic profile (with significant impact on taste) changes dramatically by malaxation temperature and time.

Are chemometrics reliable??
1st part conclusions

- All extra virgin olive oils are not the same!!
- Some oils present a great potential as health protecting or therapeutic agents
- New indexes offering an estimation of the health related properties are proposed based on NMR quantitation.
- We now have easy tools to evaluate the healthy properties of olive oil
HEALTHY POLYPHENOLS IN OLIVE OIL: WHICH ARE THE LEVELS AROUND THE WORLD?

Prokopios Magiatis, Eleni Melloul
A Key question

What levels of specific polyphenols are high, moderate or low?

The total phenolics method (Folin) cannot answer this question

To find the answer we applied the NMR method on >1500 monovarietal commercial olive oil samples from Greece, California, Italy, Spain etc for a four years period.
Greek Extra virgin Olive oil classification according to D1 and D3

Oleocanthal+oleacein = D1 index related to variety and process

Classification of Greek olive oils by origin and olive variety based on total extract H NMR

Koroneiki which represents the 70% of the olive trees in Greece gave the highest values
Variation in olecanthal content in Greek Olive oils
<table>
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<th>ΠΕΡΙΟΧΗ</th>
<th>ΕΠΟΧΗ ΣΥΓΚΟΜΙΔΗΣ</th>
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Oleocanthal/oleacin = D2 index related to variety (genetically defined)

- Oleocanthal and oleacein content of a selected group coming from Koroneiki variety from a narrow geographic origin (Messinia, Greece) showing that the ratio between both compounds is relatively stable independently from harvest time and olive mill conditions.
- In Koroneiki variety the oleacein:oleocanthal ratio was 0.56±0.19
- While in most other Greek varieties was lower 0.35±0.14
- In Arbequina or Arbosana D2 is >1
Main findings

1. There is a significant variation concerning the concentrations of oleocanthal and oleacein among the studied samples. Their concentration ranged from non-detectable to 1028 mg/Kg and their sum (index D3) from 0 to 1534 mg/Kg.

2. There are olive varieties that independently from geographic origin and harvest time produce olive oil that contains both compounds in low levels.

3. There is positive correlation of high level olive oils with the early time of harvest.
International Screening

The method was applied to 110 commercial samples found in major Supermarkets in San Francisco area.

The oils were first rapidly screened by the new selective excitation pulse method and then accurately quantified using the extraction method to undoubtedly check the health claim levels.
Geographic origin of commercial EVOO samples studied in UC Davis Olive center NOV-DEC 2013

- 48/110 California
- 26/110 Italy
- 12/110 Mediterranean mixtures
- 11/110 Greece
- 7/110 Spain
- Morocco, Argentina, Chile, France

The most expensive was from Tuscany 120$/litre. Nice taste but not health claim. Average price 15$/litre.
Comparison of supermarket samples

Concentration range in California supermarket oil produced in 2012-2013

- Oleocanthal: 0-402 mg/Kg (average 135 mg/Kg)
- Oleacein: 0-320 mg/Kg (average 105 mg/Kg)
- D3: 10-941 mg/Kg (average 330 mg/Kg)
- D4: 5-480 mg/Kg (average 174 mg/Kg)

Oleocanthal: 680 mg/Kg
Oleacein: 350 mg/Kg
Oleuropein aglycon: 53 mg/Kg
Ligstroside aglycon: 59 mg/Kg
Total hydroxytyrosol derivatives (D4): 403 mg/Kg
D3: 1142 mg/Kg

Values of the best Greek bottled EVOO 2013-2014
Results

50% of the samples showed total tyrosol and hydroxytyrosol derivatives >250 mg/Kg

22 out of 110 showed hydroxytyrosol derivatives (oleacein+oleuropein aglycon) > 250 mg/Kg (5 Italy, 1 Spain, 1 Greece, 15 California)

It is estimated that about 20% of the olive oil brands available in supermarkets can support the EU health claim
Interesting: D3 can predict organoleptic properties.

In collaboration with TEI of Kalamata we correlated the sensory evaluation results with the D3 and we were able to predict the bitterness and pungency based on the oleocanthal and oleacein content for EVOO.

\[ y = 0.0055x + 0.2683 \]
\[ R^2 = 0.7517 \]
Health claims=better price?

The olive oils that contain high quantities of hydroxytyrosol and its derivatives will be able to claim health protecting activities and consequently to achieve a better price.

Patients with cardiovascular diseases = an new consumer target for olive oil

However the consumers must be educated to accept and value the taste of these oils.

It will also be a difficult task for the producers to achieve balanced taste and high concentration of specific polyphenols.
NMR is the most powerful tool for olive oil quality evaluation and especially for the health claim proof.

Our target is to make the qNMR method officially accepted by EU or IOOC.

Currently World Olive Labs (Richmond, Virginia) is the only provider of this specific analysis in the US and the University of Athens in Europe.
THANKS TO

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