



1. Use of Health Claims / EU Regulation/ Phenolic content measurement

2. Parameters affecting the presence of polyphenols in EVOO before the Olive mill (Variety, harvesting period, irrigation, diseases, olive collection, terroir)
3. Parameters affecting the presence of polyphenols in EVOO at the Olive mill (mill type, malaxation and separation conditions)
4. Parameters affecting the presence of polyphenols in EVOO after the Olive mill (Filtration, storage conditions, packaging)



Project co-financed by the European Regional Development Fund



Mediterranean diet can save your life

From Ancient Greece

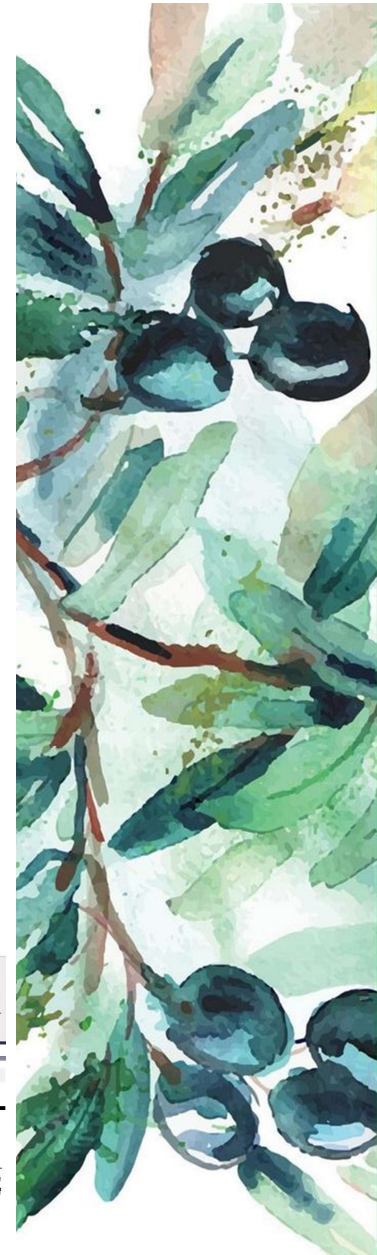


Latest News

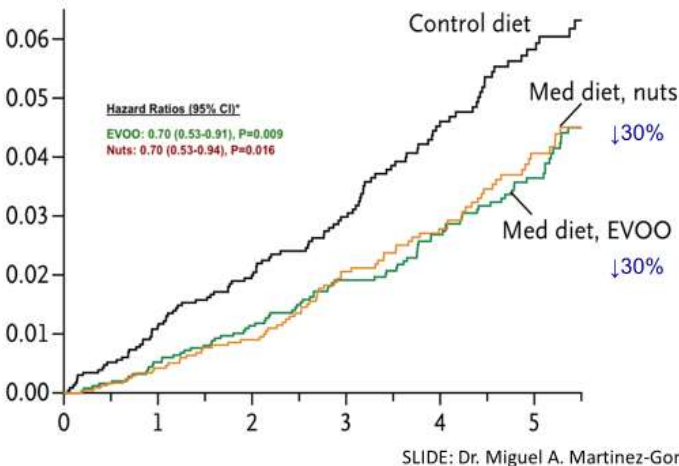


Dioscorides

- 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



Predimed: Risk of heart attack, stroke, CV death



SLIDE: Dr. Miguel A. Martinez-Gonzalez

Adherence to Mediterranean diet and risk of cancer: an updated systematic review and meta-analysis of observational studies

Lukas Schwingshackl & Georg Hoffmann

Cancer Medicine 2015; 4(12): 1933–1947

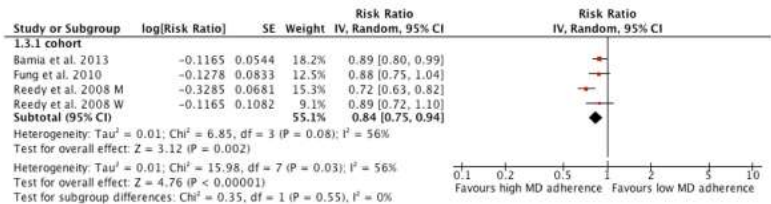
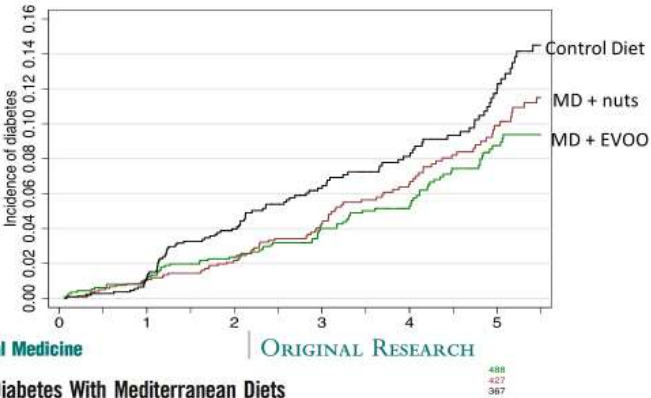


Figure S2. Forest plot showing pooled risk ratio (RRs) with 95% CI for risk of colorectal cancer for three cohort studies, and four case-control studies.

SLIDE: Dr. Estefania Toledo

Predimed: Prevention of diabetes

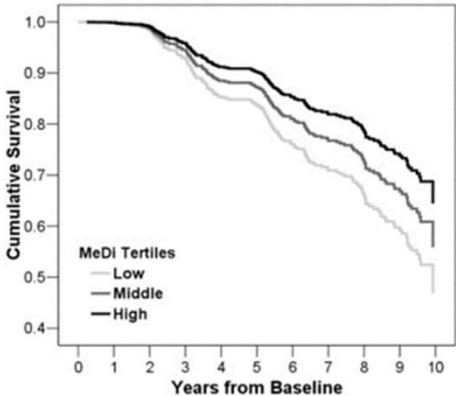


Prevention of Diabetes With Mediterranean Diets A Subgroup Analysis of a Randomized Trial

Jordi Salas-Salvadó, MD, PhD*; Mónica Bulló, PhD; Ramón Estruch, MD, PhD; Emilio Ros, MD, PhD; María-Isabel Covas, DPharm; Núria Izquierdo-Jurado, RD, PhD; Dolores Corella, DPharm, PhD; Fernando Arós, MD, PhD; Enrique Gómez-Gracia, MD, PhD; Valentina Ruiz-Gutiérrez, PhD; Dora Romaguera, MD, PhD; José Lapetra, MD, PhD; Rosa María Lamuela-Raventós, DPharm, PhD; Lluís Serra-Majem, MD, PhD; Xavier Pons, MD, PhD; Josep Basora, MD, PhD; Miguel Ángel Muñoz, MD, PhD; José V. Sorlí, MD, PhD; and Miguel A. Martínez-González, MD, PhD*

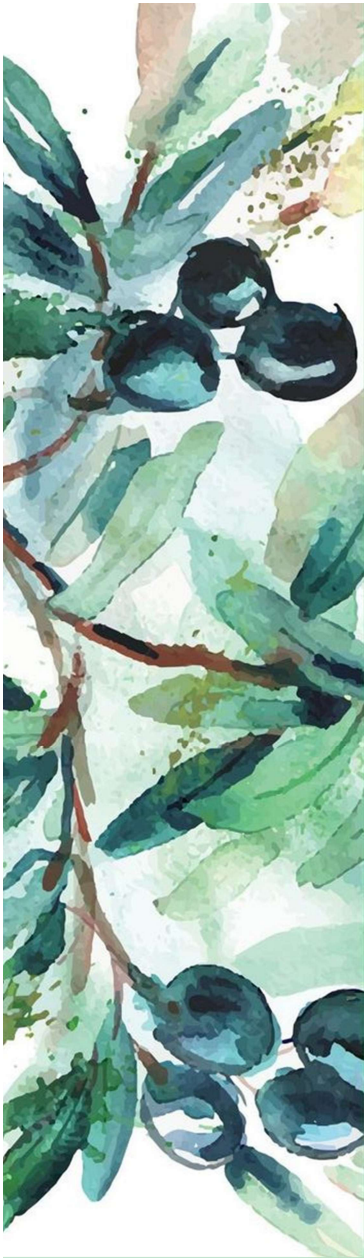
SLIDE: Dr. Miguel A. Martinez-Gonzalez

Mediterranean Diet and Alzheimer’s dementia



Scarmeas, Luchsinger, Annals Neurol 2006

SLIDE: Dr. Jose A. Luchsinger

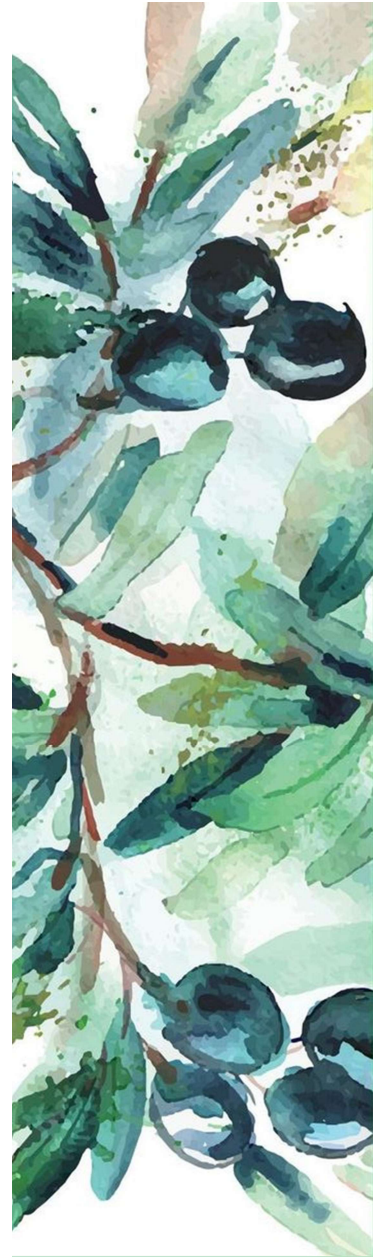


Food or drug?

Olive oil is not a simple food or a simple source of lipids

- It contains healthy monounsaturated lipids BUT it also 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

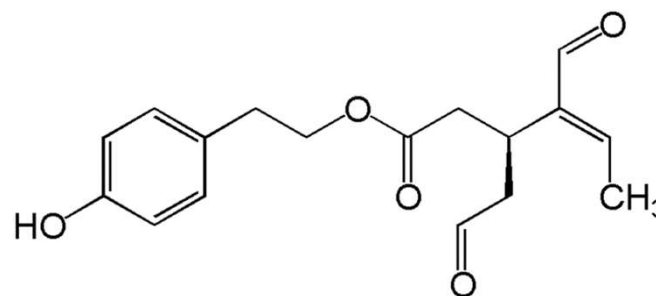
Olive oil is in the **intermediate** between food and drug providing an excellent example of Hippocratic medicine



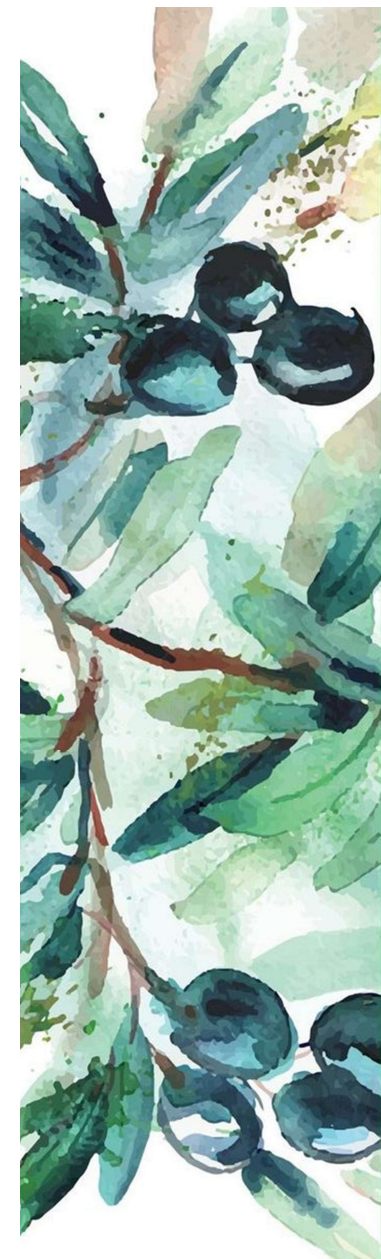
Minor chemical compounds

- Oleocanthal (decarboxymethyl ligstroside aglycone) is responsible for the pungency of fresh olive oil **coming from unripe** olives (irritation of oropharyngeal region).
- It disappears from the oil as the fruit matures
- Oleocanthal possesses antiinflammatory activity similar to Ibuprofen as a COX-2 inhibitor

Oleocanthal



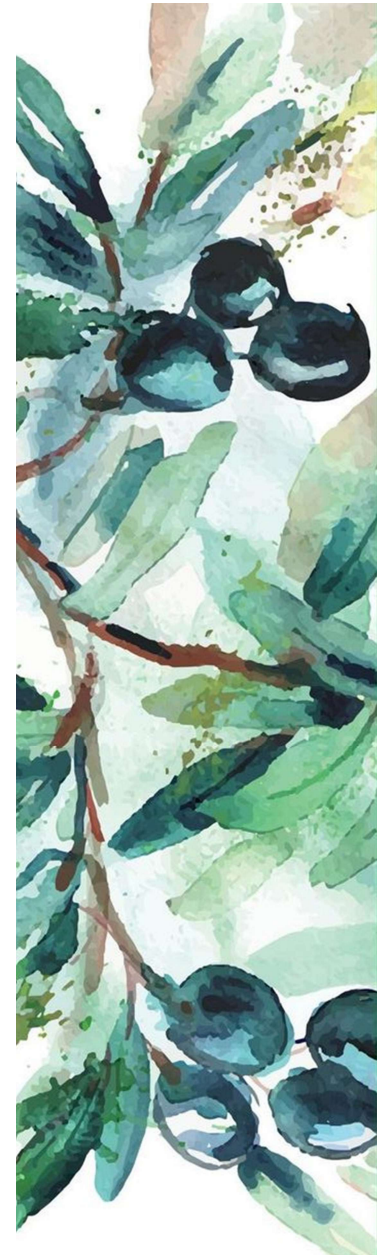
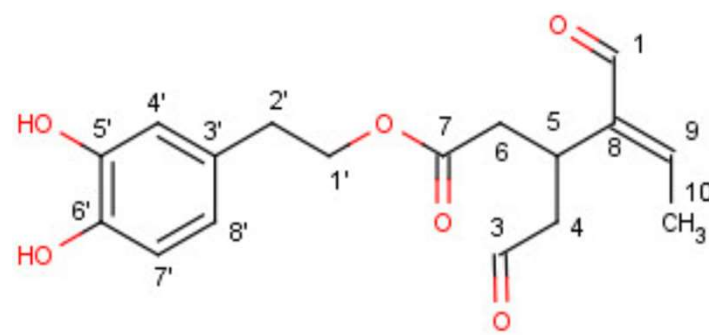
"Phytochemistry: Ibuprofen-like activity in extra-virgin olive oil."
Beauchamp, Gary K. et al. **Nature** (2005), 437(7055), 45-46



Minor chemical compounds

- Oleacein has a similar structure with oleocanthal.
- It is a derivative of hydroxytyrosol
- It is the most powerful antioxidant constituent of olive oil and also an inhibitor of LOX

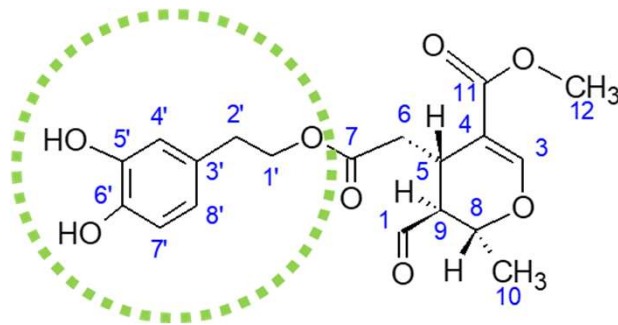
Oleacein



Minor chemical compounds

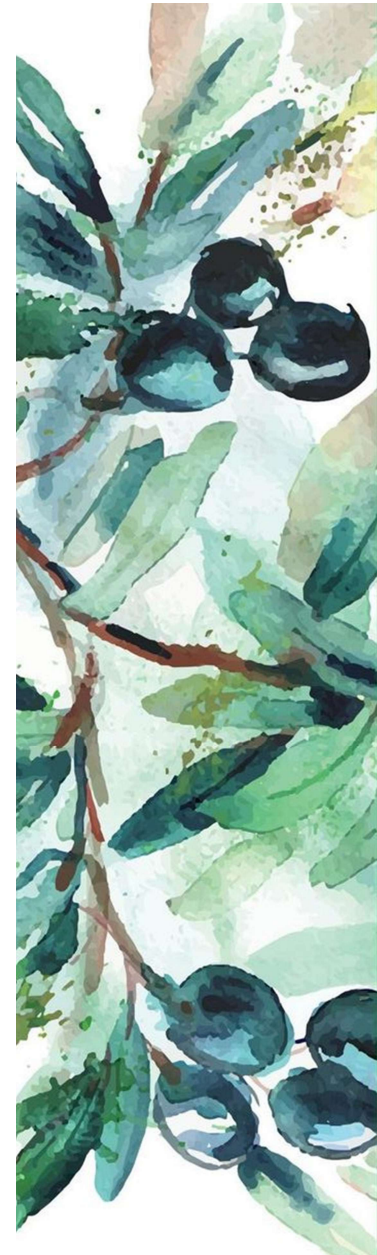
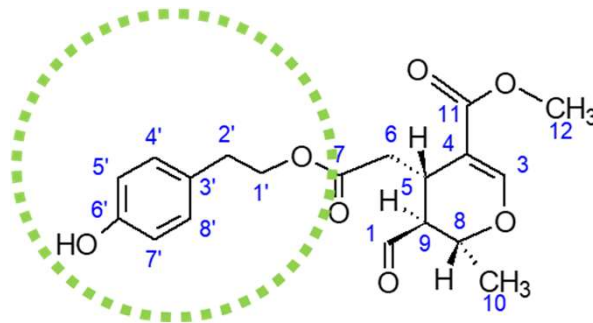
Oleuropein aglycon (monoaldehyde form)

- Powerful antioxidant
- Protection against Alzheimer in animals



Ligstroside aglycon (monoaldehyde form)

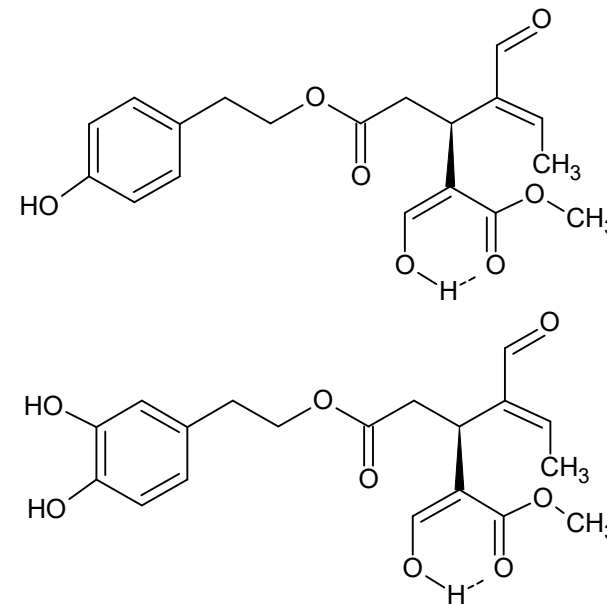
- Antioxidant activity
- Can block metastasis



Minor chemical compounds

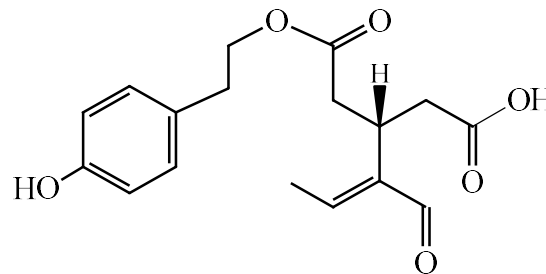
Oleokoronal-Oleomissional

- 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
- Reduced bitterness
- bioactivity under investigation



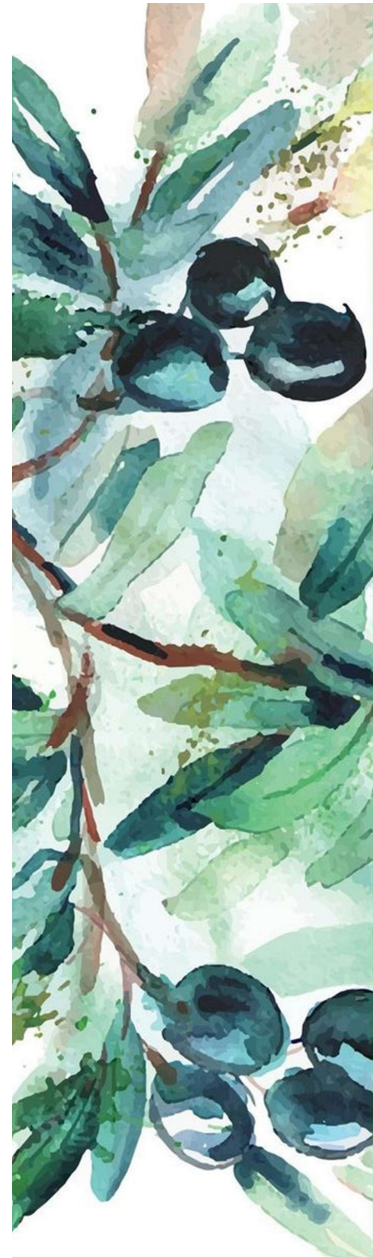
Oxidized derivative of oleocanthal

- Neuprotective activity



What is the official status of olive oil?

- Olive oil is not yet recognized as a normal medicine or as a traditional herbal medicine with therapeutic use by EMA or FDA.
- It is recognized by EFSA and by FDA as a food with qualified health claims (under specific conditions related to chemical composition)
- In this frame it can be used as ingredient of food supplements distributed through the Pharmacy stores
- However there are numerous experimental studies that demonstrate the therapeutic properties of specific types of olive oil or of specific ingredients.



How EFSA and FDA regulations work?

Europe-EFSA

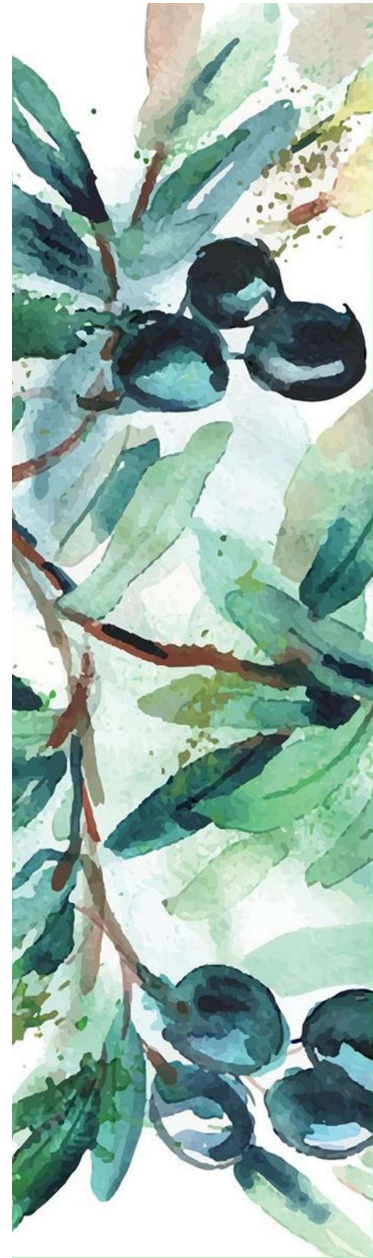
Food supplements are concentrated sources of nutrients (or other substances) with a nutritional or physiological effect that can be marketed in "dose" form, such as pills, tablets, capsules, liquids in measured doses, etc.

USA-FDA

Dietary supplements. A dietary supplement is either intended to provide nutrients in order to increase the quantity of their consumption, or to provide non-nutrient chemicals which are claimed to have a biologically beneficial effect.

Dietary supplements should not be used to treat any disease or as preventive healthcare

Health claims on food labels and in food marketing are claims by manufacturers of food products that their food will reduce the risk of developing a disease or condition



Qualified health claims of Olive oil

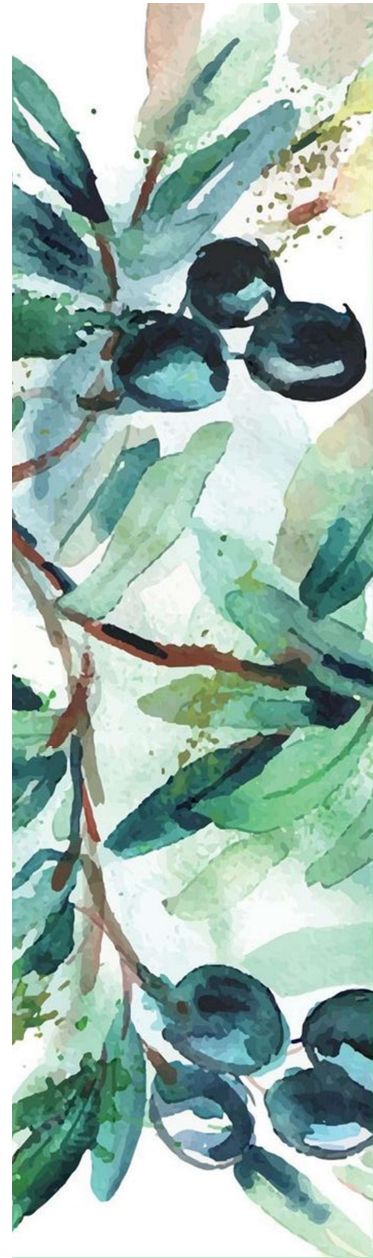
FDA:

Limited and not conclusive scientific evidence suggests that eating about 2 tablespoons (23 grams) of olive oil daily may reduce the risk of coronary heart disease due to the monounsaturated fat in olive oil. To achieve this possible benefit, olive oil is to replace a similar amount of saturated fat and not increase the total number of calories you eat in a day.

EFSA:

- Replacing saturated fats in the diet with unsaturated fats contributes to the maintenance of normal blood cholesterol levels. Oleic acid is an unsaturated fat.
- Vitamin E contributes to the protection of cells from oxidative stress
- **Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress**

The claim may be used only for olive oil which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil.



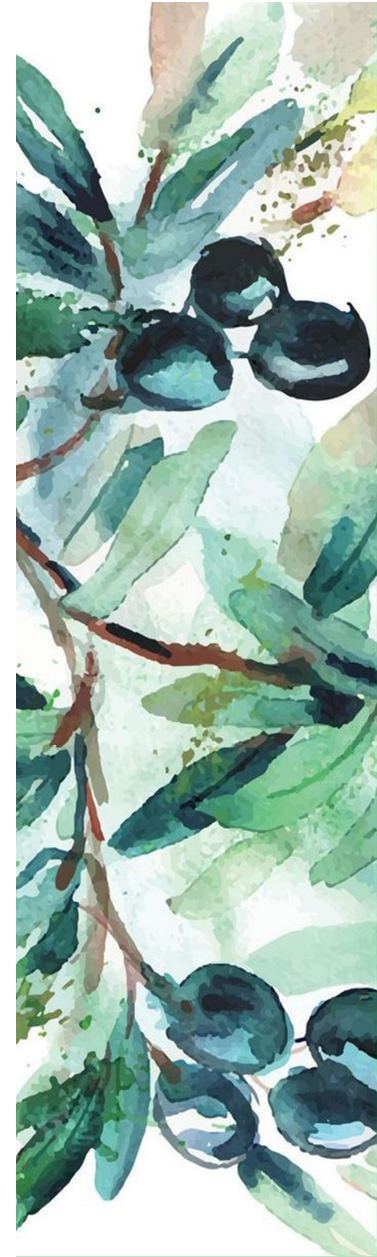


The EU regulation: A closer look

- 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.

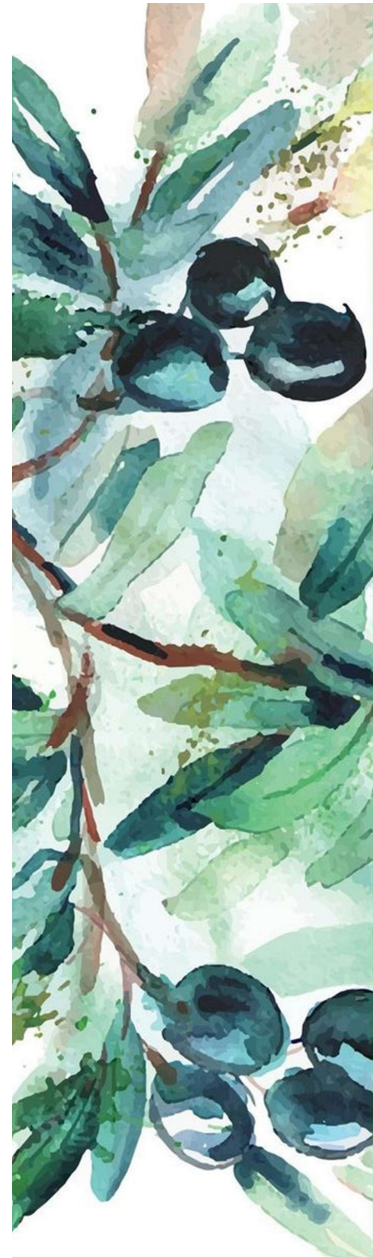
A few years ago we published a reliable method to measure all the compounds mentioned in the regulation in one experiment and provide the necessary data for the health claims

Magiatis et al JAFC 2014

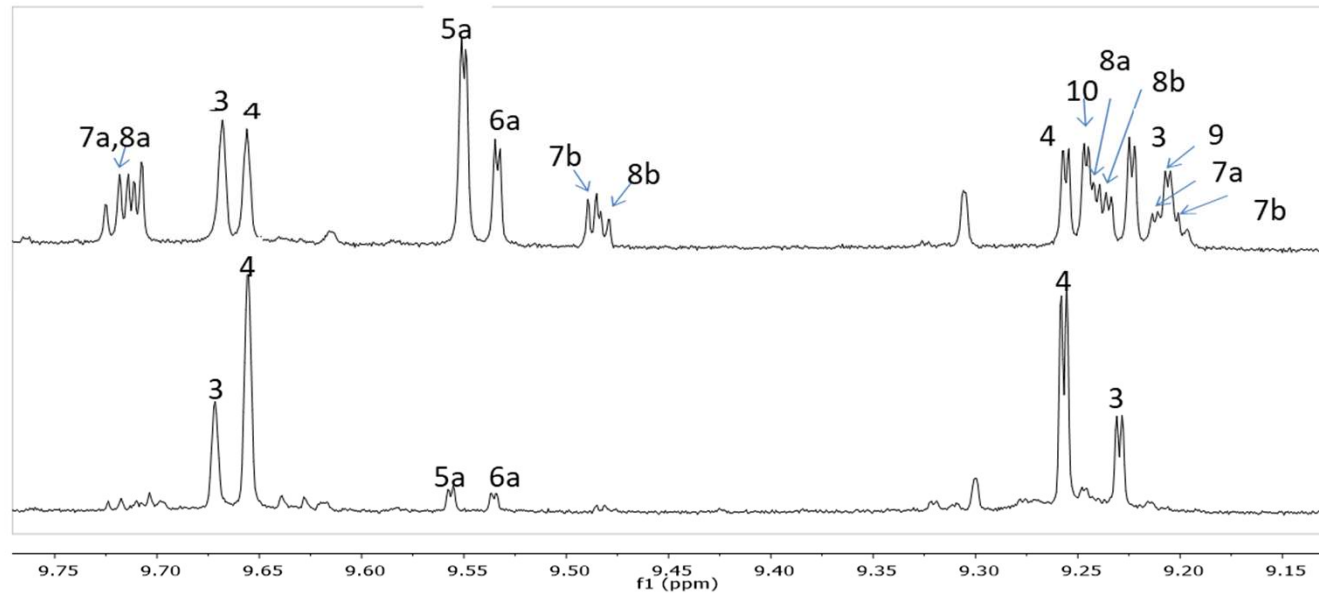


Need for certified EVOOs with polyphenol health claim

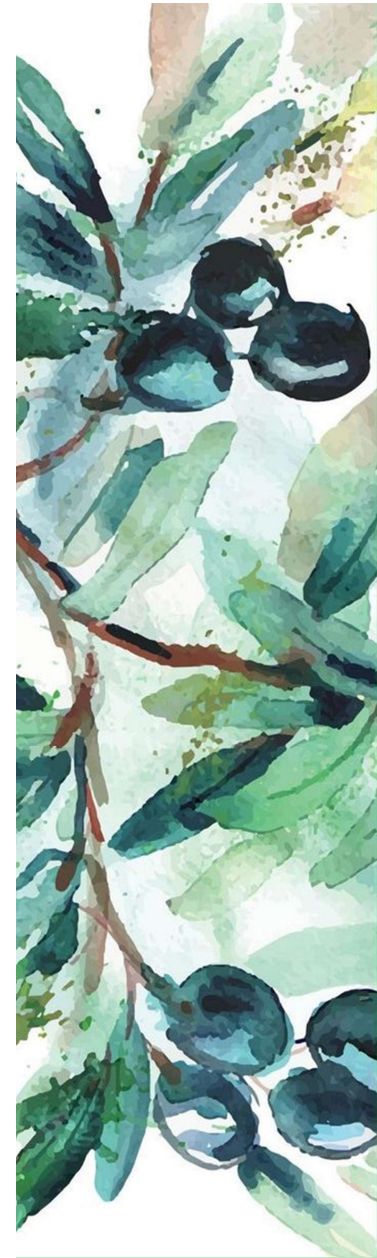
- Organoleptic evaluation is not enough
- HPLC-UV method of IOC quantify phenols as tyrosol and hydroxytyrosol equivalents and FOLIN methods as gallic or caffeic acid equivalents which leads in underestimation of total phenolic content.
- The health claim can be certified by precise measurement of all the phenols included in the health claim regulation using laboratory methods like the qNMR.
- As a result of Aristoil project we published an improved reliable method to measure all the compounds mentioned in the regulation in one experiment and provide the necessary data for the health claims based on NMR method.

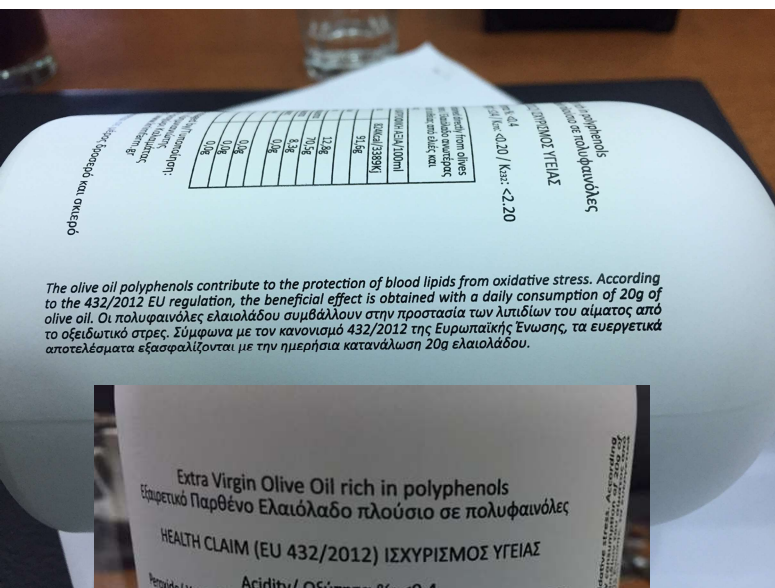


A typical example of an NMR spectrum

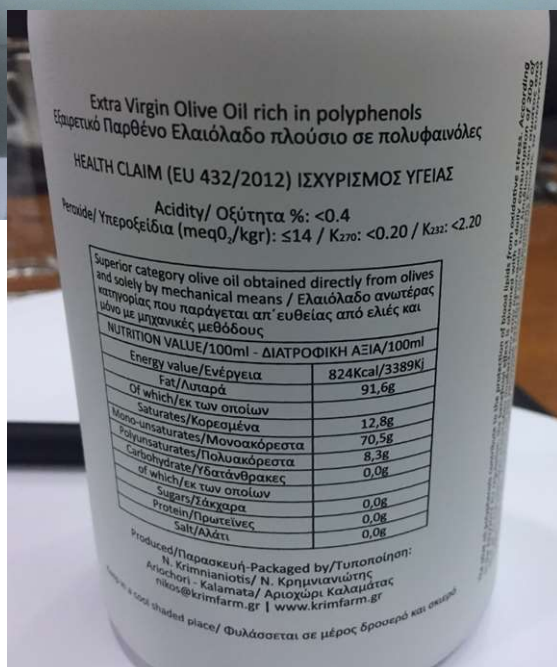


- With this method we are able to measure each phenolic constituent independently
- Avoiding any misleading results





The olive oil polyphenols contribute to the protection of blood lipids from oxidative stress. According to the 432/2012 EU regulation, the beneficial effect is obtained with a daily consumption of 20g of olive oil. Οι πολυφαινόλες ελαιολάδου συμβάλλουν στην προστασία των λιπιδίων του αίματος από το οξειδωτικό στρες. Σύμφωνα με τον κανονισμό 432/2012 της Ευρωπαϊκής Ένωσης, τα ευεργετικά αποτελέσματα εφασφαλίζονται με την ημερήσια κατανάλωση 20g ελαιολάδου.



The first olive oil in Greece approved as food supplement



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PhenOLIV Protect®



Product Description:

PhenOLIV Protect is a Natural Food Supplement using the Extra Virgin Olive Oil (EVOO) from our trees with the highest level of polyphenols. Over the past years we have refined our harvesting and production process to greatly increase the level and profile of polyphenols in our olive oil. Some of the most important polyphenols (oleocanthal and others) are "created" during the olive mill process and are not available in other forms of olive based supplements.

The EVOO we use for PhenOLIV Protect is carefully analysed and the polyphenols are preserved in the most natural way to ensure that our olive oil has a high level of polyphenols throughout its life. Based on its natural ingredients PhenOLIV Protect contributes to:

- the protection of blood lipids from oxidative stress
- the protection of cells from oxidative stress
- the maintenance of normal blood cholesterol levels
- the normal functioning of the immune system

In order for the benefits to apply 10ml (9.15g) of PhenOLIV Protect should be consumed daily.

Food supplements should not be used to replace a healthy balanced diet. Recommended daily allowance should not be exceeded without first consulting your doctor. This product is not intended for the treatment of diseases. Consult your doctor if you are pregnant, breast feeding or taking medication. Store in a cool dry place away from humidity. Keep away from children. The expiry date is only valid for products with intact packaging and stored correctly.

Poison Control Number: +30 210 7793777

National Organisation for Medicines Notification Number: 111258/11-12-2017

The Notification Number does not require a circulation number from the National Organisation for Medicines

Available in: 200ml bottles with dosometric lid.

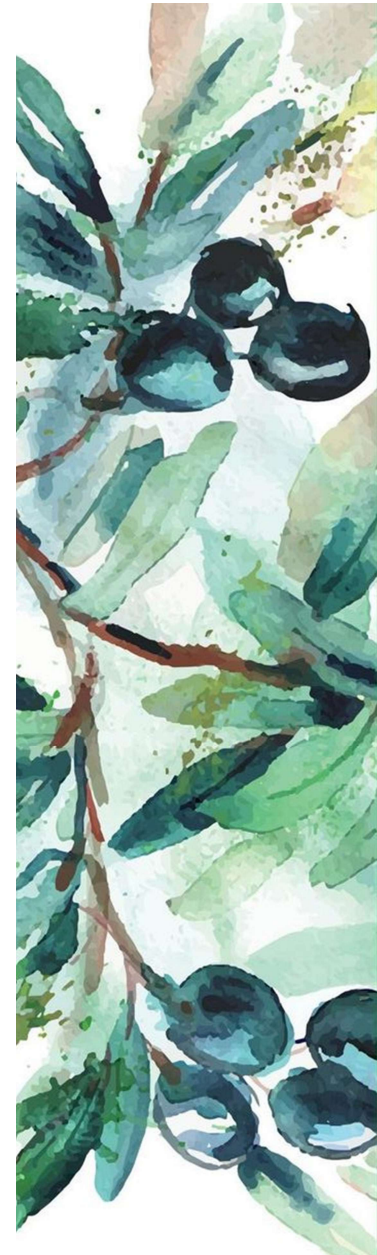
Connection of price with phenolic content

The phenolic content should be related with the price because:

- A 250 mg/Kg oil requires consumption of 20 g to offer the necessary amounts of phenolics
- A 2500 mg/Kg oil requires only 2 g to offer the same amount of healthy phenolics with less calories
- A reasonable connection must be adopted by the market

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.

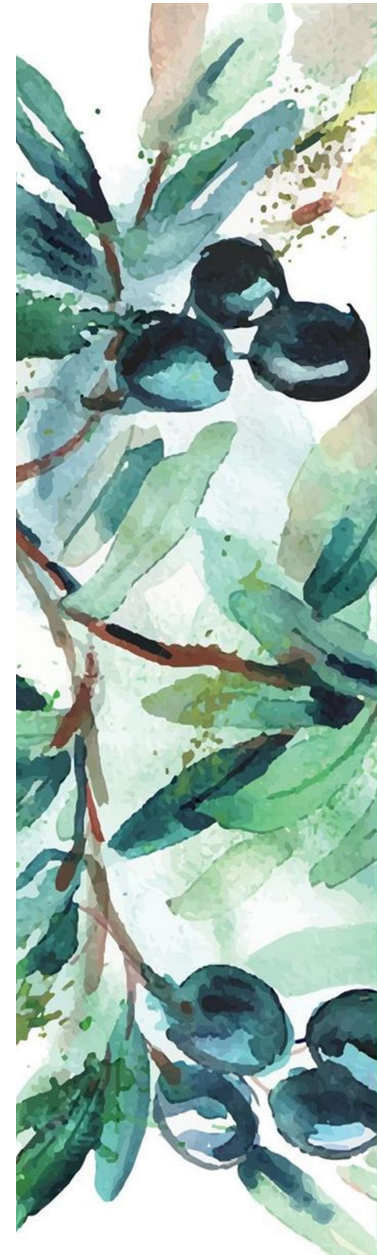
Patients with **cardiovascular, metabolic or neurodegenerative diseases** = an new consumer target group for olive oil



How to build a high phenolic Brand



- The consumers need to be informed about the health protecting properties of olive oil.
- The consumers must be educated to accept and value the taste of these oils.
- It will also be a challenging task for the producers to achieve balanced taste and high concentration of specific polyphenols.
- We must encourage millers to move towards this direction





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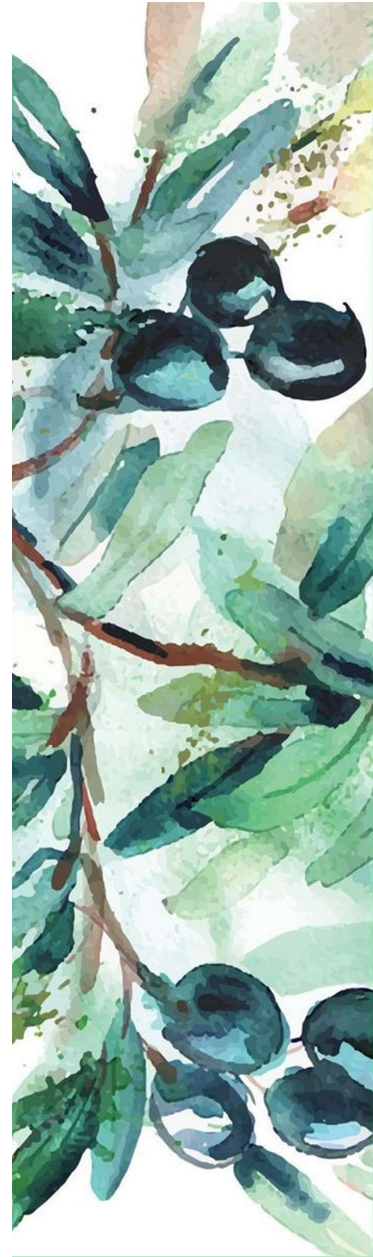


Parameters affecting the presence of polyphenols in EVOO

The olive fruit cultivar and olive oil production is a very complex process

Many factors affect the quality of the final product.

- Variety
- Pedoclimatic Conditions
- Irrigation
- Harvest
- Olive fruit transfer to the mill
- Olive oil extraction Process
- Storage



Crucial Parameters that affect the presence of polyphenols in EVOO

Olive fruit variety



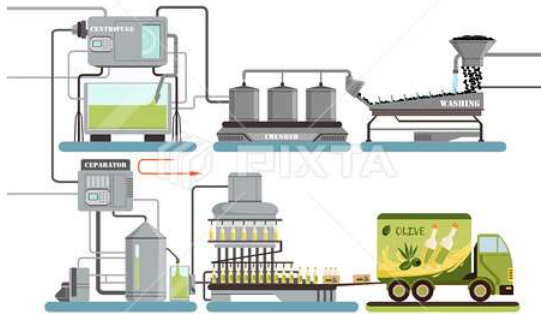
Harvest Period



Transfer to olive mill

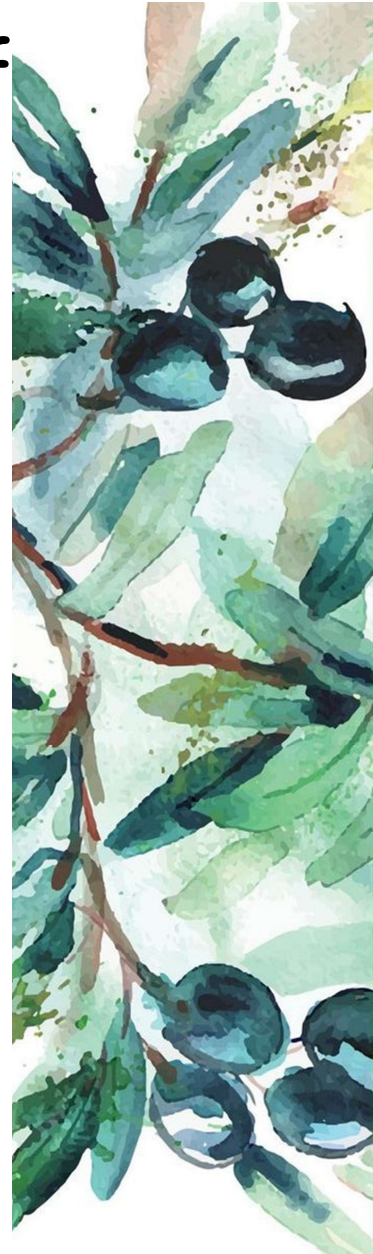


Olive mill Process



pixtastock.com - 64060785

Storage



Olive fruit variety

- More than one thousand olive varieties worldwide
- Growing in more than 23 Countries.

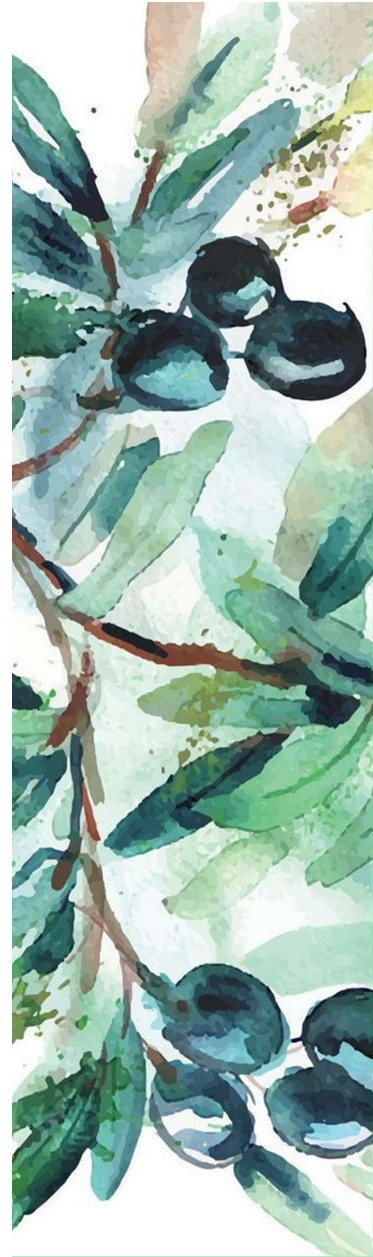
Are all these varieties similar as it concerns the phenolic content of the producing olive oil ?

No

Is it possible to produce a high phenolic olive oil from any variety?

Yes

The answers were revealed during **Aristoil Project**



Olive fruit variety

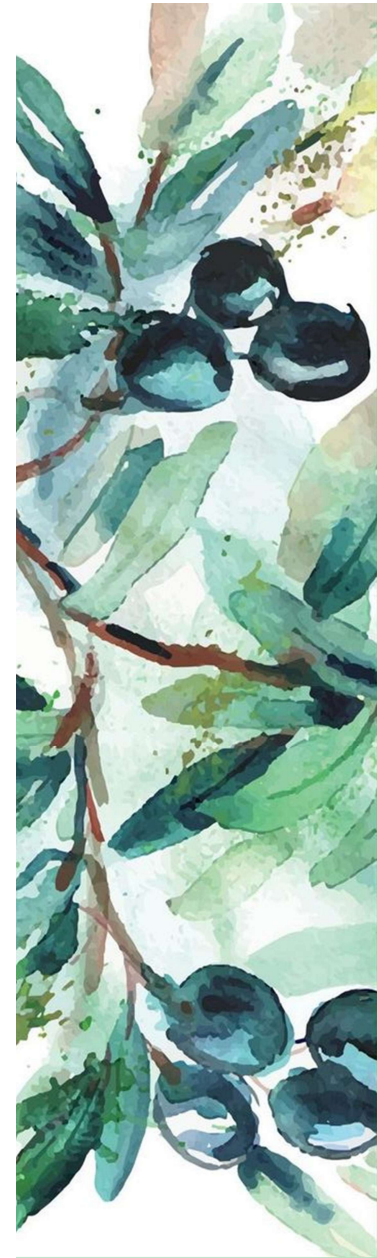
- We studied 19 different varieties from Greece.
- Some of them are very common in Greece while some others were really rare.
- Among the 13 most common varieties:

There were varieties with really high average of total phenolic content

Even varieties with low average phenolic content could produce olive oil with extremely high phenolic content

Average phenolic content of different varieties

Variety	n	Average	maximum
Olympia	213	895	2,343
Lianolia	283	710	2,442
Kalamon	29	589	1,898
Athinolia	228	560	1,712
Chalkidiki	312	477	1,468
Amfissa	235	425	1,438
Wild	142	411	2,382
Koroneiki	2,183	404	2,079
Mourtolia	13	384	1,287
Kolovi	34	382	1,204
Koutsourelia	166	344	1,059
Megaritiki	65	246	849
Manaki	222	239	1,396

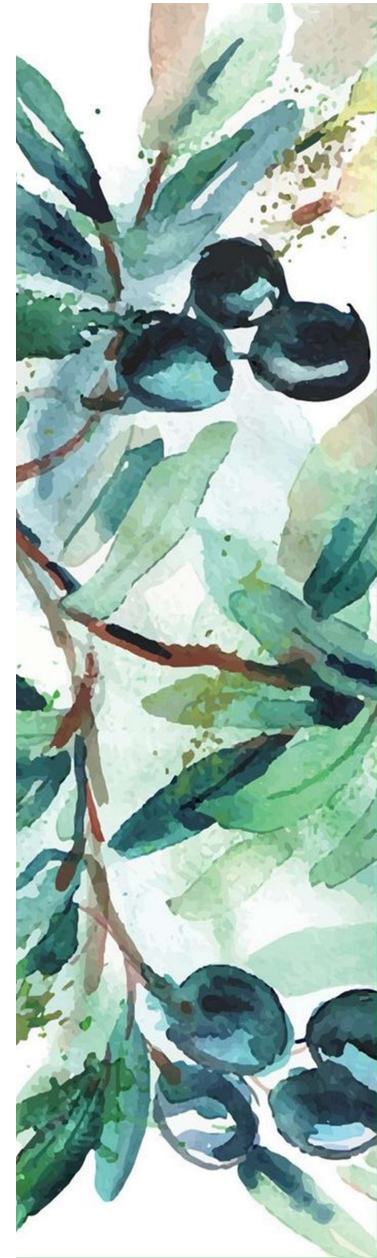


Olive fruit variety

- There are some varieties which are easier to produce a high phenolic olive oil
- There are other varieties which are more difficult to achieve this target
- In most of the varieties the average of hundreds of samples could use the EU regulation for the Health claim.

Variety	n	Subset							
		1	2	3	4	5	6	7	8
Olympia	260	986.4							
Zakynthou	44		880.0						
Kalamon	59			769.9					
Lianolia Kerkyras	350			704.9					
Athinolia	260				585.2				
Chalkidikis	362					486.1			
Agrielia (wild)	164					444.0	444.0		
Koroneiki	2649					434.3	434.3		
Amfissas	276					414.7	414.7		
Kolovi	40						378.3		
Koutsourelia	189						364.8	364.8	
Megaritiki	81							285.0	285.0
Manaki	261								259.6

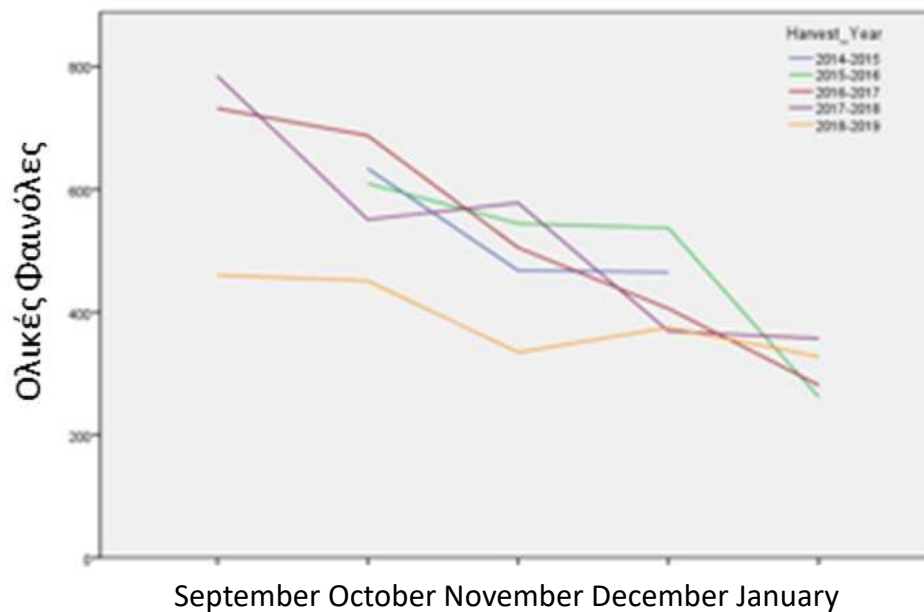
In order to achieve this goal other parameters like olive fruit transfer to the olive mill, olive oil extraction or harvest period should be examined



Olive fruit Harvesting

Olive fruit harvesting is one of the most crucial factors that affect the concentration of phenols in olive oil

- After the analysis of thousands of samples it was obvious that in the early stages of ripening total phenols are higher than in the late ripening.
- The olive oil producer should take under consideration the increase of total phenolic content and the price of his product but also the decrease of the oil yield

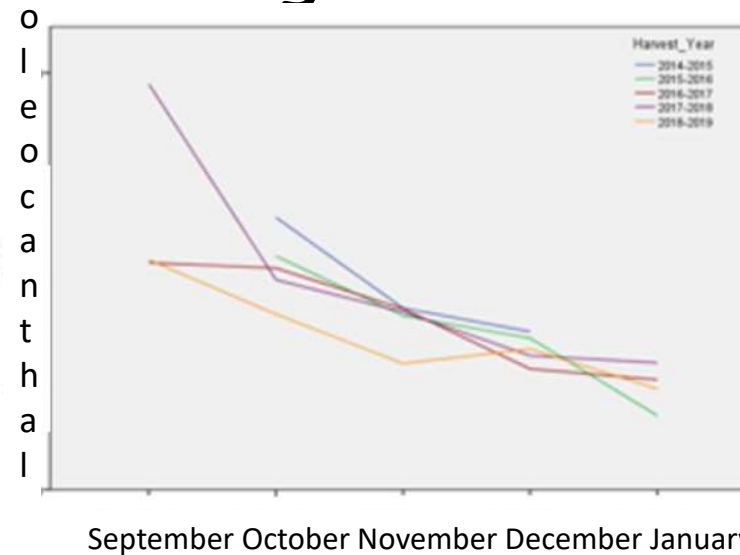


Olive fruit Harvesting

The same motive is also followed by the individual phenols and especially for oleocanthal

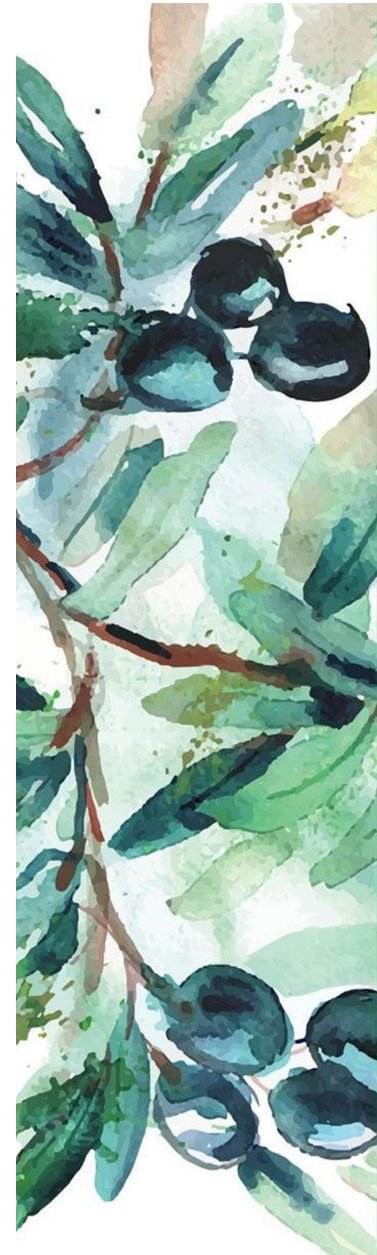
Harvesting in early ripening offers

- Lower acidity, peroxide and K index values
- Higher phenolic content



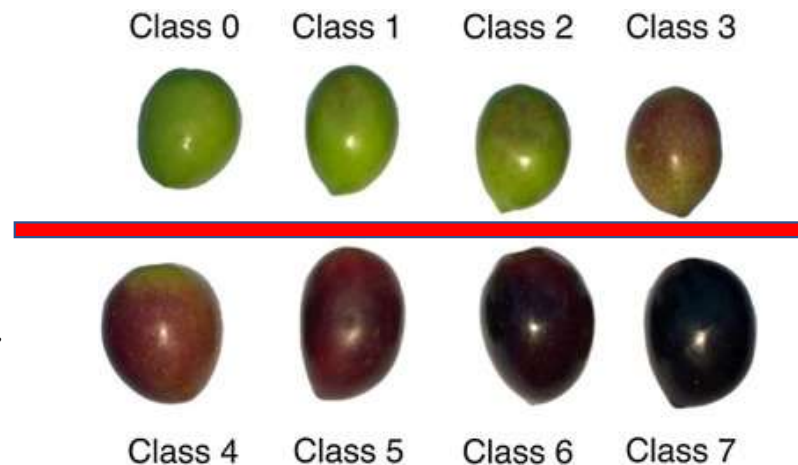
Comparison of mean concentration of oleocanthal (mg/kg) among different months

Month of harvest	n	1	2	3	4
September	111	251.06			
October	613		191.34		
November	1017			159.35	
December	864				131.84
January	72				111.95



Olive fruit Harvesting

- Each variety follows similar but different ripening rate
- The climatic conditions differ from year to year.
- Its important for the producers to choose the appropriate time to harvest their orchard.
- For many varieties (but not all) the maturity Index is a safe way to choose the appropriate date.

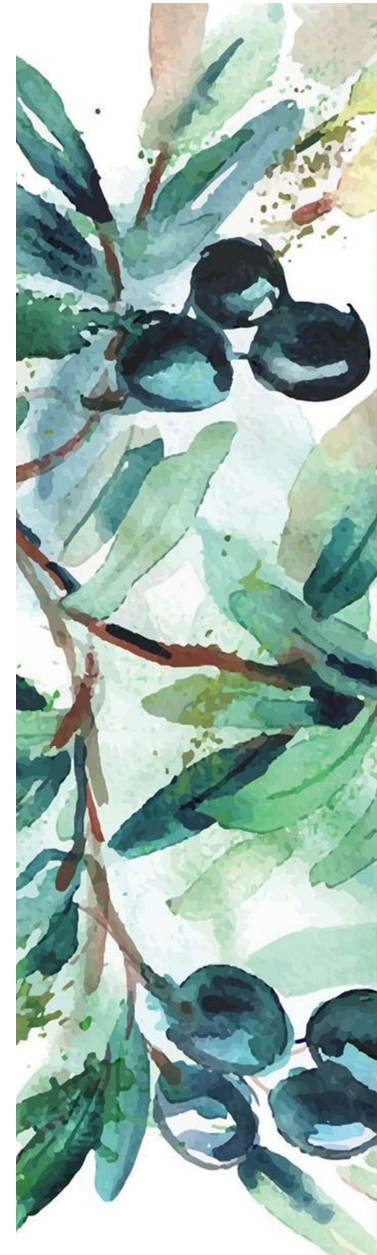


Always choose to pick olive fruits while green color is still visible



Olive fruit transfer to the olive mill

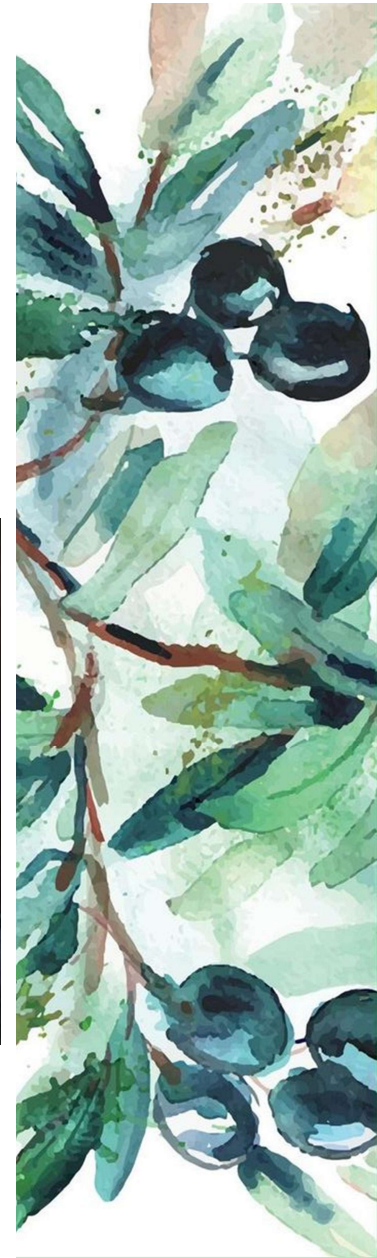
- Producers should avoid sacks for the fruit storage
- And definitely the use of plastic sacks is forbidden
- Producers should prefer open crates in order to preserve the quality of the olive fruit till milling.



Olive fruit Storage

Olive oil producers should minimize the time between harvesting and milling

- Apart from the type of storage (sacks, crates etc)
- Olive fruits should be stored less than 24 hours before milling
- Longer storage affects negatively on the phenolic content and the rest quality parameters.





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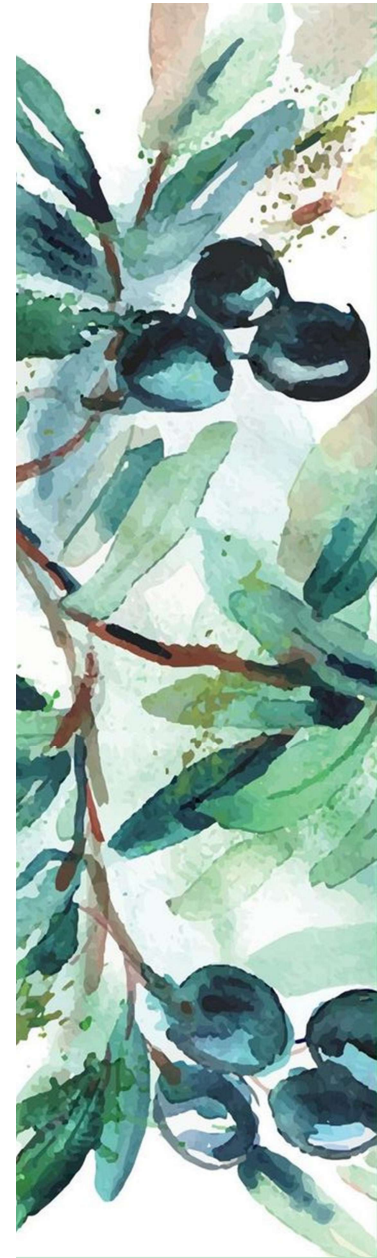
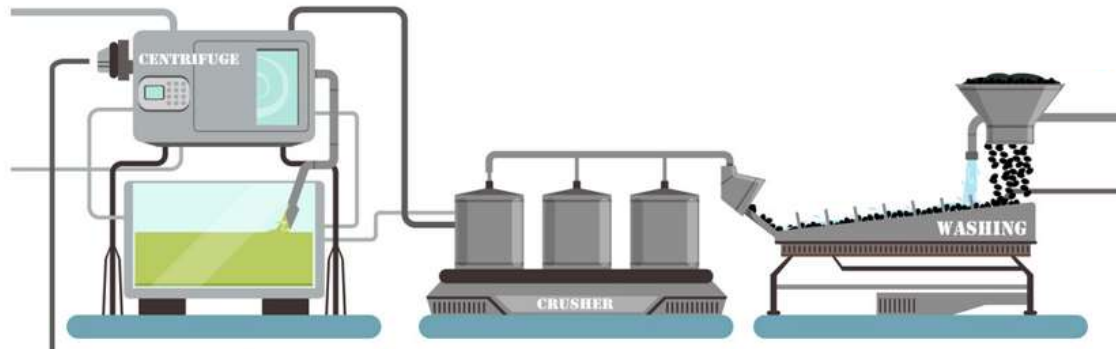


At the Olive Mill

The first part of the production of olive oil has to do with the cultivation, harvest, even the transportation of the olive fruit to the olive mill



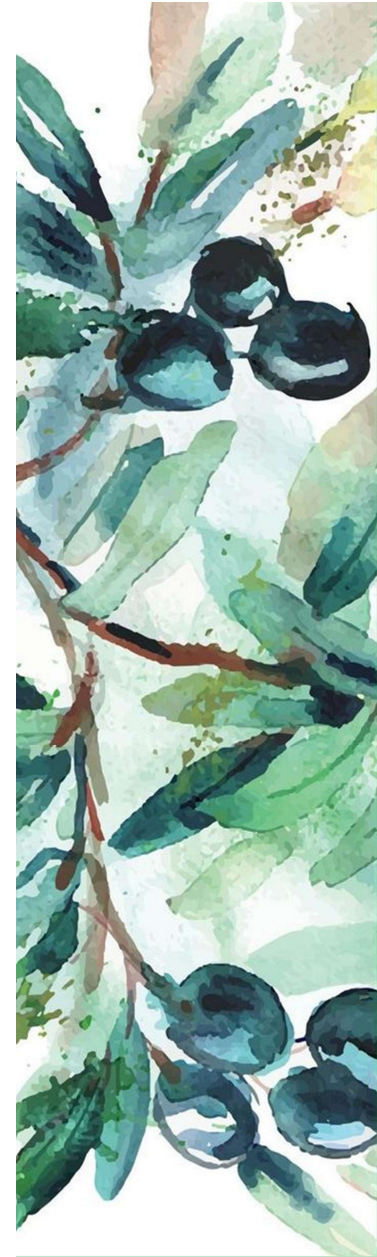
Even if the producer has applied the best practices to upgrade the quality of his product in the first step, everything can change during the second part of the production of olive oil which is the extraction process



Olive Mill

Olive oil production

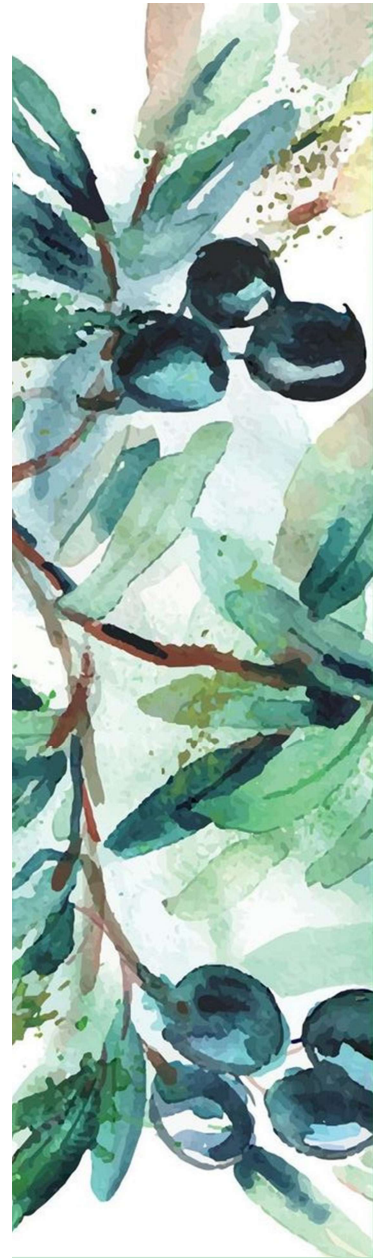
- In the olive mill the olive fruit is cleaned with air and water
- The olive fruit is getting crushed
- And the olive paste is getting malaxed under heat for 15-75 mins.
- Olive oil is collected by horizontal followed by vertical centrifugal separation.



Malaxation process

The malaxation process is important for the production of high phenolic olive oil because:

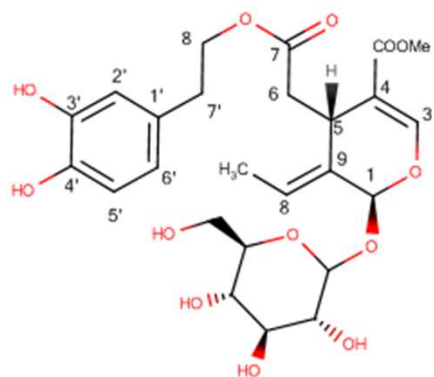
- It gives time to break the cell walls and release olive oil droplets inside the paste
- Allows the smaller droplets of oil to be released and aggregate and be more easily separated.
- It gives time for the production of the fat soluble phenols and their solubilization in olive oil
- All these goals are enhanced by the application of heat.



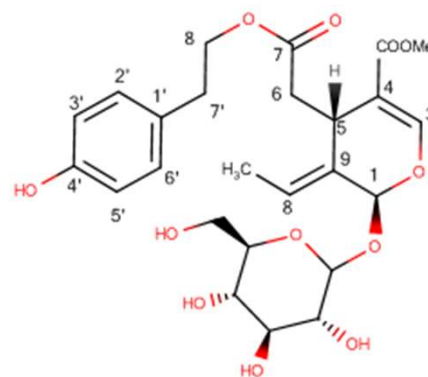
How phenols are made

- Olive oil phenols are absent from the olive fruit
- In the olive fruit we can only find small quantities of tyrosol and hydroxytyrosol as a result of the degradation of Oleuropein and Ligstroside
- In the olive fruit the major phenolic constituents are Oleuropein and Ligstroside

Oleuropein



Ligstroside

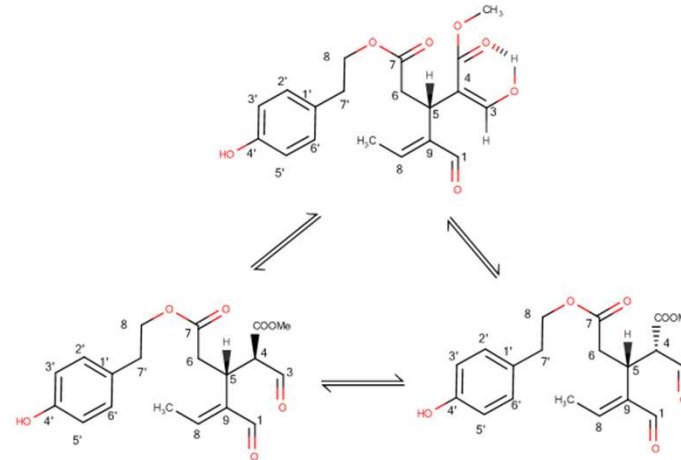
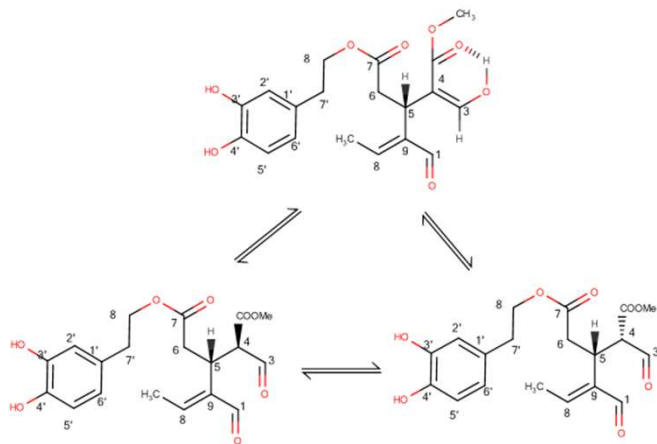
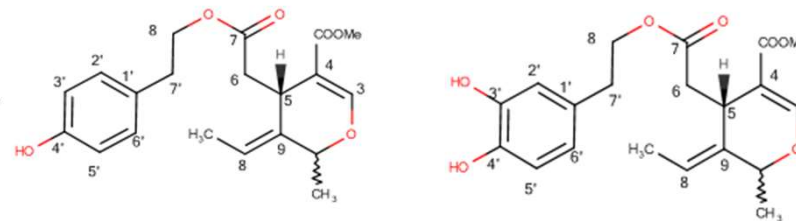
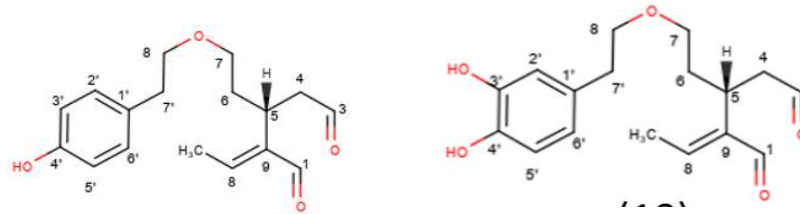


- These are the precursors of all the phenolic constituents we find in EVOO



How phenols are made

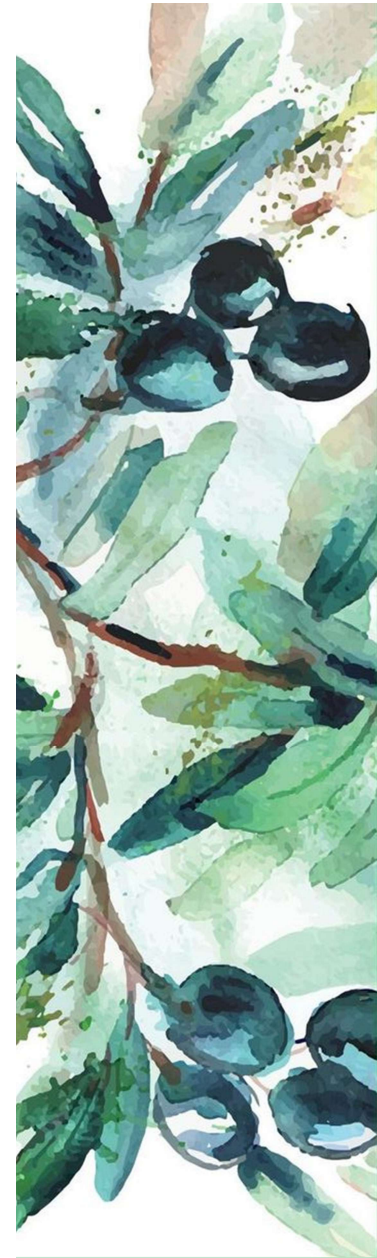
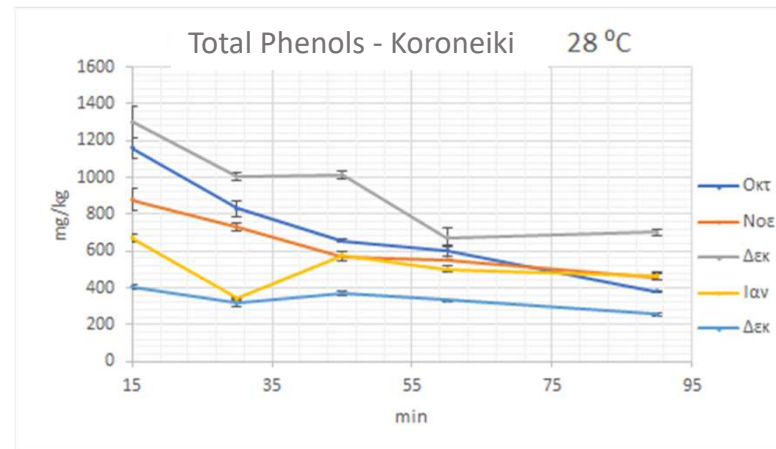
- During olive fruit crushing and malaxation of the olive paste a number of enzymes transform these two constituents
- All the phenolic constituents are produced during these process.
- At the same time Hydrolytic and oxidati enzymes perform degradation of all phenolic constituents



Malaxation Time

Talking about enzymes we should know that their activity is strongly related by the duration and the temperature of the process.

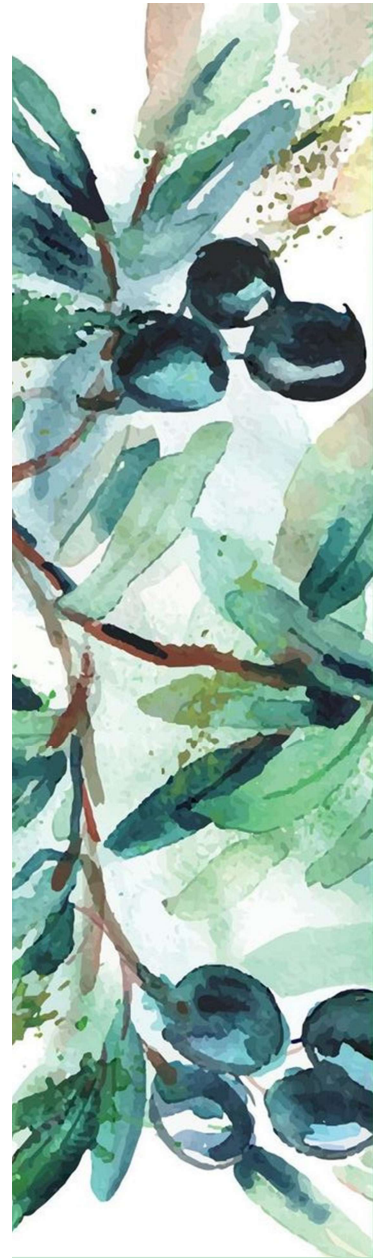
- After crushing there is the highest content of phenolic compounds
- After this point the total phenolic content is decreased.
- The Total phenolic content could be **decreased** by more than **70%**



Malaxation Time

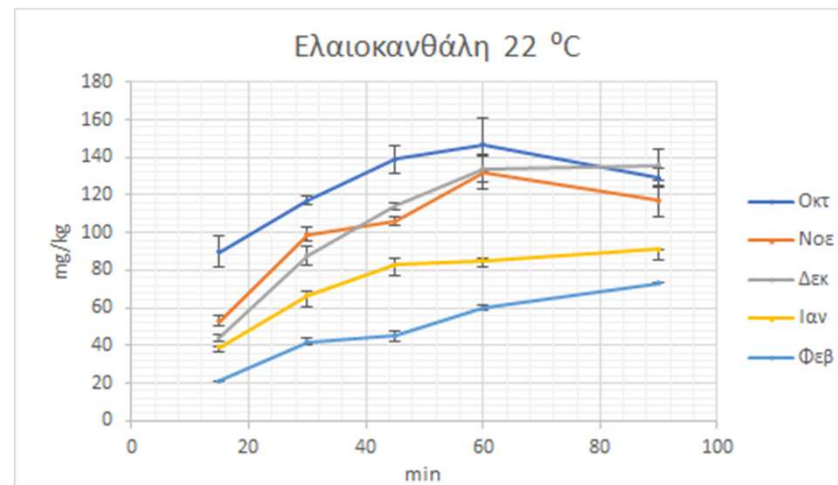
- The maximum amount of the total phenolic content is determined by all the factors we discussed before.
- But the malaxation step can change the result dramatically
- The same olive fruits can give a totally different result.

Hydrolytic enzymes may harm olive oil during malaxation



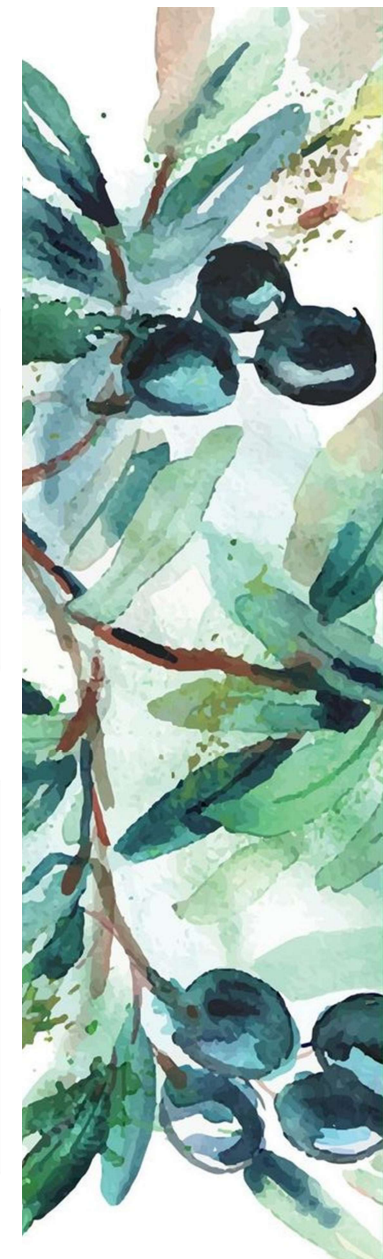
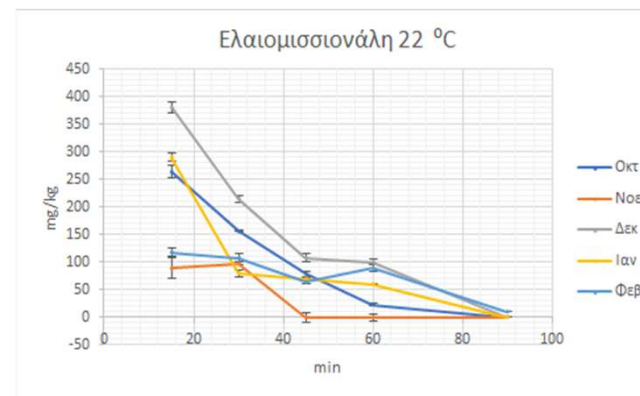
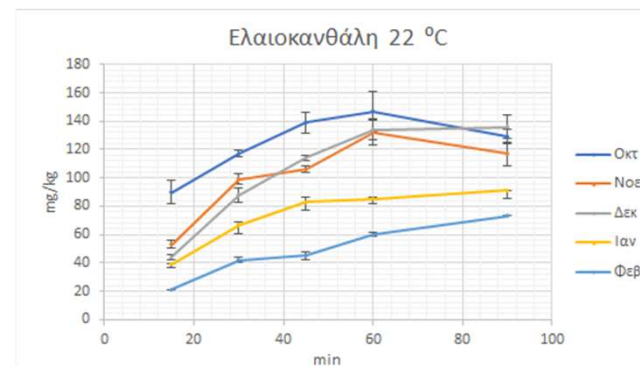
Malaxation Time

- On the other hand, the activity of some enzymes leads to the increase of the production of oleocanthal and oleacein.
- These two constituents need some time to be produced
- These two constituents are very important for olive oil as they determine the final organoleptic and health protective characteristics of olive oil.



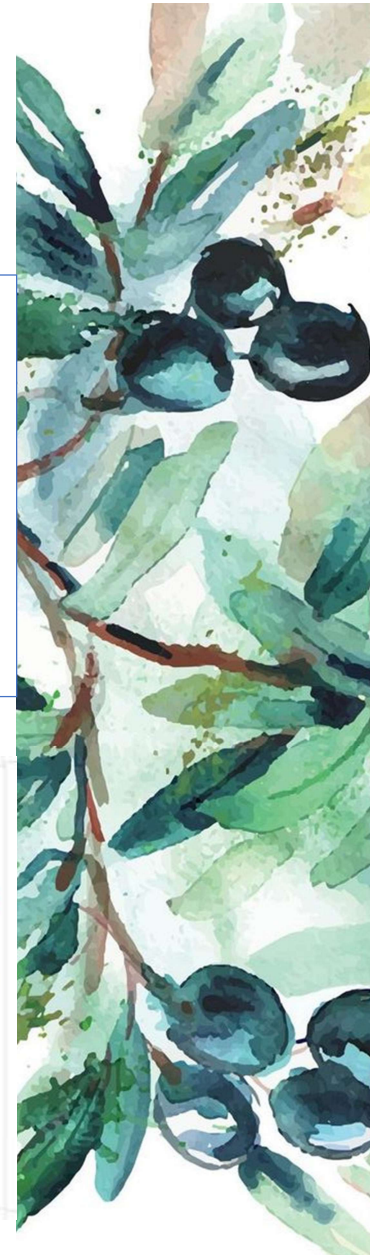
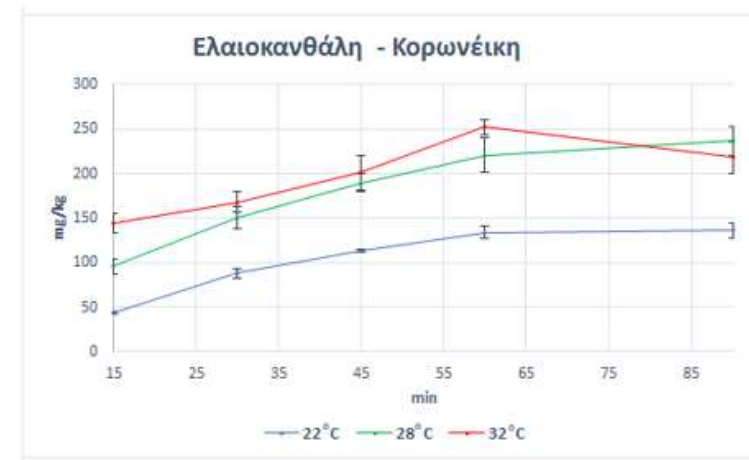
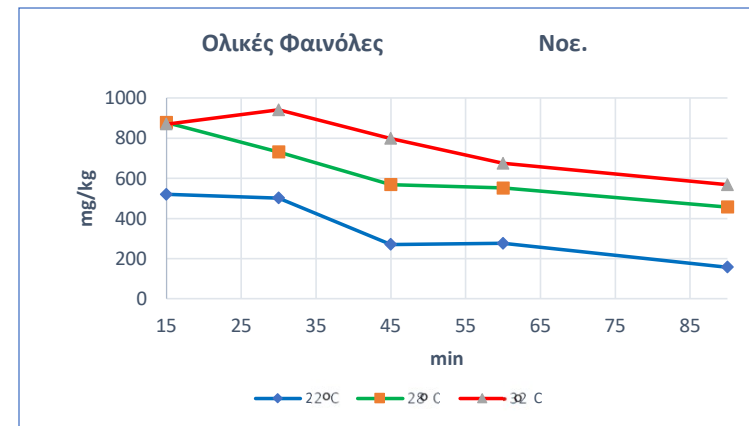
Malaxation Time

- While total phenolic content is decreasing during malaxation
- A class of phenolic constituents is decreasing and at the same time another class (oleocanthal and oleacein) are increasing concentration.
- Some enzymes perform the transformation of the precursor constituents to oleocanthal and oleacein.
- This transformation is achieved in different time depending the variety of the olive fruit.



Malaxation Temperature

- As we mentioned all the phenolics are produced during malaxation and under enzyme activity
- It is known that enzyme activity is enhanced by temperature around 28- 30 °C
- The appropriate temperature for the production of a high phenolic olive oil is 28- 30 °C
- This will accelerate the formation of oleocanthal and oleacein



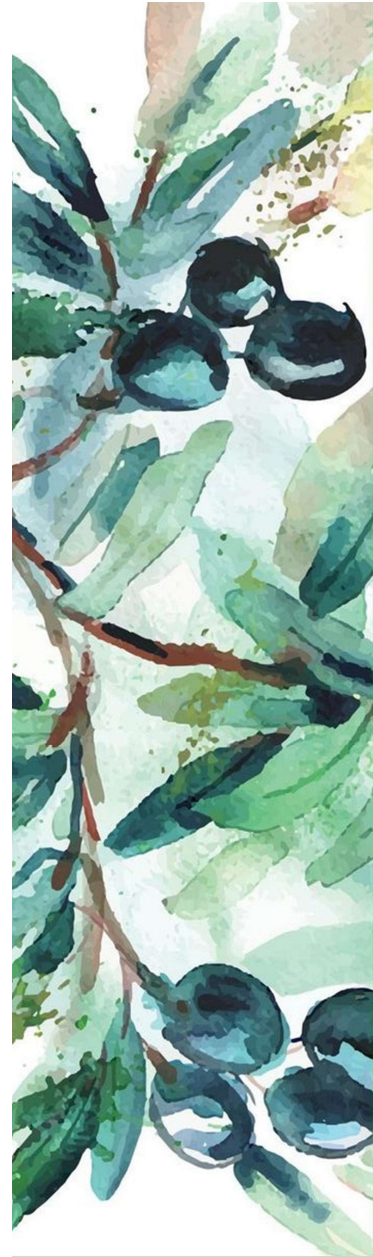
Malaxation Temperature

BUT

- Apart from the enzymes which produce phenols
- There are hydrolytic and oxidative enzymes which result degradation of phenols

So

- Increase of temperature
- should be followed by decrease of malaxation time



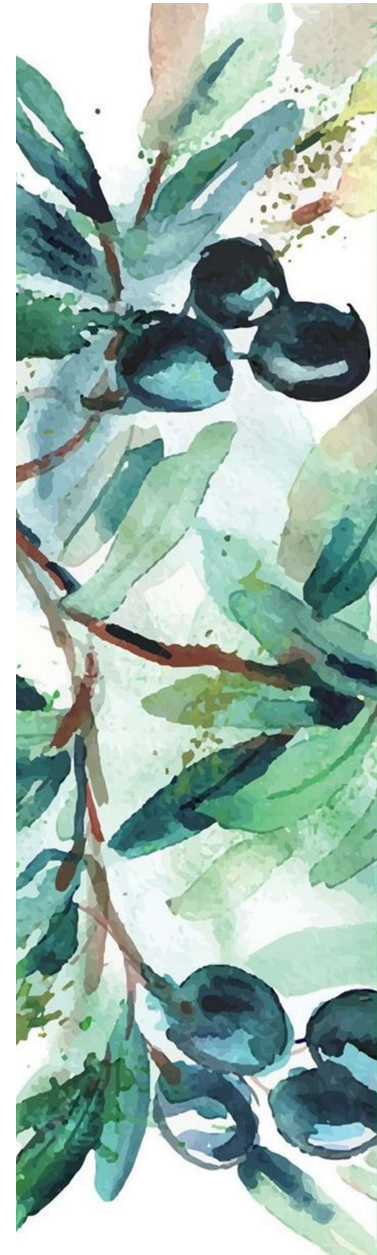
Olive oil production

- Olive paste centrifugation in decanters is mandatory for olive oil rapid extraction
- Olive oil cleaning with water is usually applied in centrifuges
- This process result the instant mixing of olive oil with water which degrades phenols.

The use of water in every step should be avoided or kept at a minimum.

Two-phase mills should be preferred instead of three phase

If the second vertical centrifugation cannot be avoided, then is strictly recommended to avoid hot water





1. Use of Health Claims / EU Regulation/ Phenolic content measurement
2. Parameters affecting the presence of polyphenols in EVOO before the Olive mill (Variety, harvesting period, irrigation, diseases, olive collection, terroir)
3. Parameters affecting the presence of polyphenols in EVOO at the Olive mill (mill type, malaxation and separation conditions)
4. **Parameters affecting the presence of polyphenols in EVOO after the Olive mill (Filtration, storage conditions, packaging)**

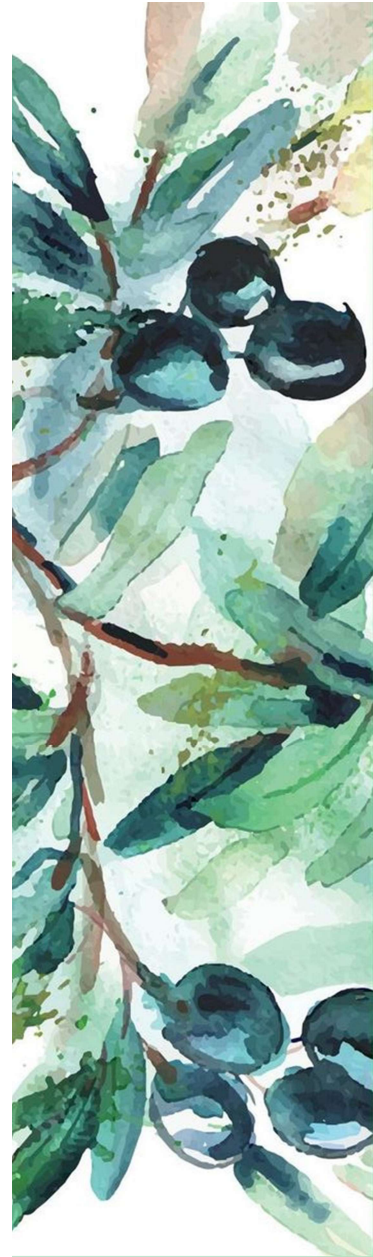


Project co-financed by the European
Regional Development Fund



Storage & Packaging

- It is the final step for the olive oil production.
- It is also crucial for the maintenance of the organoleptic characteristics of olive oil
- The phenolic content maintenance and
- Determines the shelf life of the product.

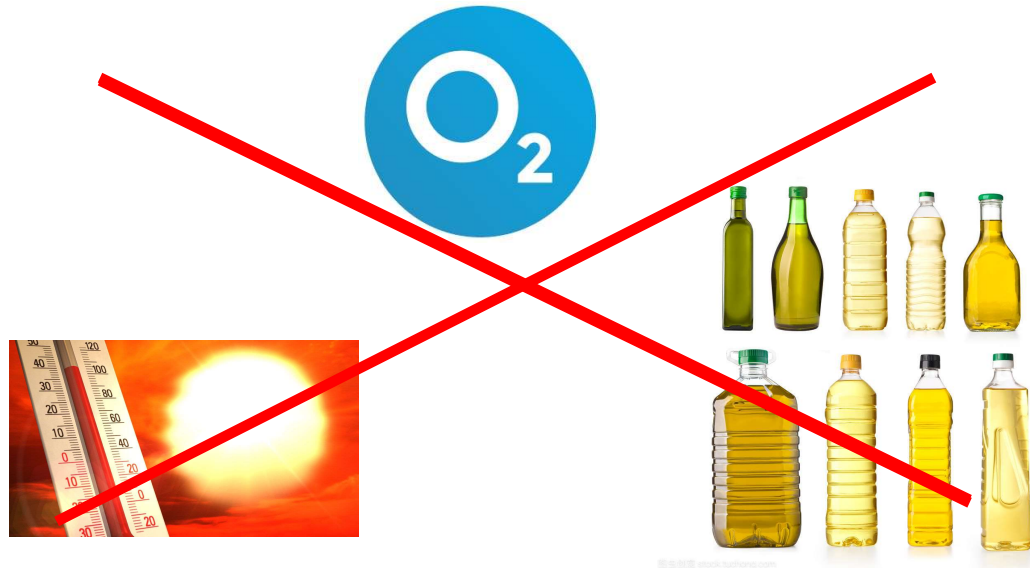


Storage & Packaging

In storage and packaging line there are some factors that affect dramatically the quality of olive oil.

Olive oil producers should avoid the prolonged contact with:

- Atmospheric Oxygen
- Residual Water
- Heat
- Light (Plastic bottles)



Filtration

- After olive oil production for some days the product is cloudy
- Cloudy olive oil has been established as a trademark of a fresh olive oil

BUT

Olive oil is cloudy because of impurities and humidity which remains in fresh olive oil for some days after its production

These impurities are harmful for the phenol content and the organoleptic characteristics of olive oil

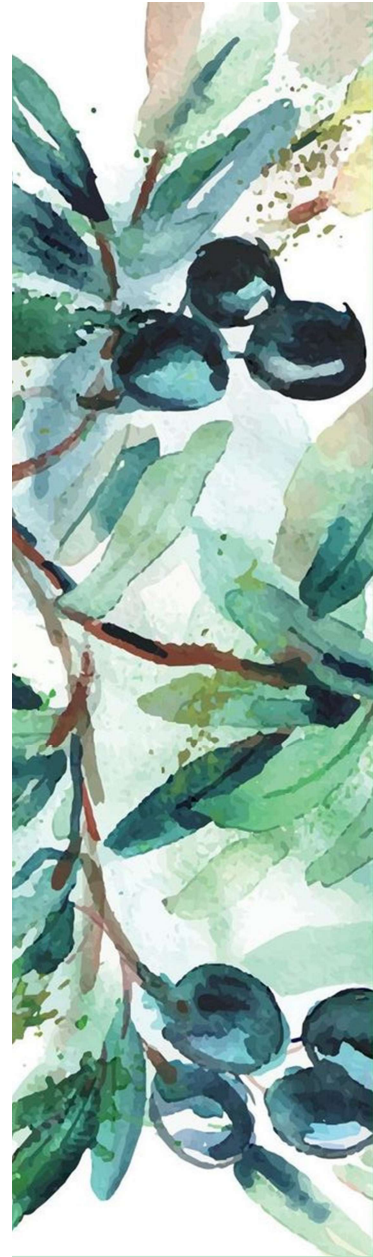
Olive oil filtration as soon as possible



Storage of olive oil.

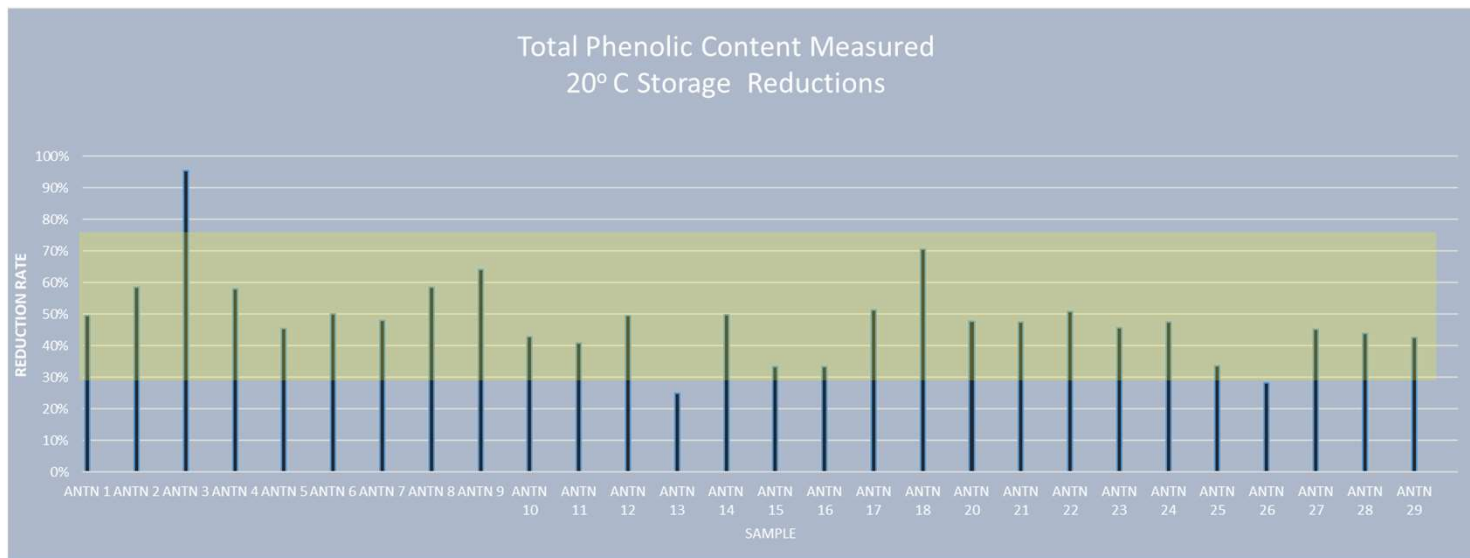
After olive oil production and filtration of olive oil Olive oil producers should :

- Store olive oil in inox tanks with the minimum headspace
- If possible fill head space with N_2 or Ar.
- Remove often impurities from the bottom of olive oil.
- Keep olive oil in a steady temperature under $18\text{ }^{\circ}\text{C}$



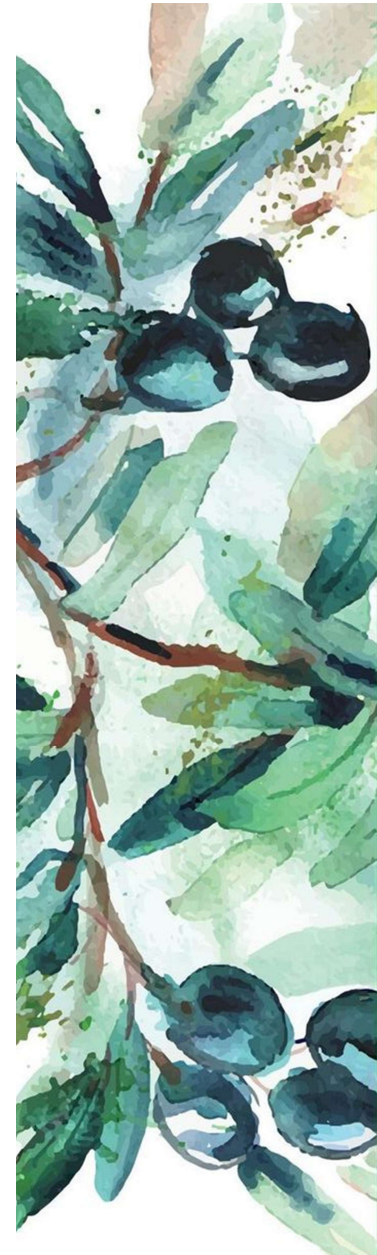
Storage of olive oil.

- Low temperature (4°C) will cause less than 15% degradation of total phenols in 12 months
- Room Temperature will cause approximately 30-50% after 12 months



Packaging of olive oil.

- Avoid plastic bottles for the packaging of olive oil.
- Glass bottle are preferable but still this is not enough.
- Olive oil should be packaged in dark bottles to avoid light harm your product.
- Bag- in box is the best type of packaging
- Always leave the minimum head space





National and Kapodistrian
University of Athens,
Faculty of Pharmacy, Greece



RECENT STUDIES ON THE BIOLOGICAL EFFECTS OF THE POLYPHENOLS OF OLIVE OIL

Recent Studies on The biological effects of the polyphenols of Olive Oil

Prokopios Magiatis

Interreg
Mediterranean



ARISTOIL PLUS

Project co-financed by the European
Regional Development Fund

What is the official status of olive oil?

- ★ Olive oil is not yet recognized as a normal medicine or as a traditional herbal medicine with therapeutic use by EMA or FDA.
- ★ It is recognized by EFSA and by FDA as a food with qualified health claims (under specific conditions related to chemical composition)
- ★ In this frame it can be used as ingredient of food supplements distributed through the Pharmacy stores
- ★ Or as ingredient of cosmetics
- ★ However there are numerous experimental studies that demonstrate the therapeutic properties of specific types of olive oil or of specific ingredients.



What is
the
opinion
of the
market?

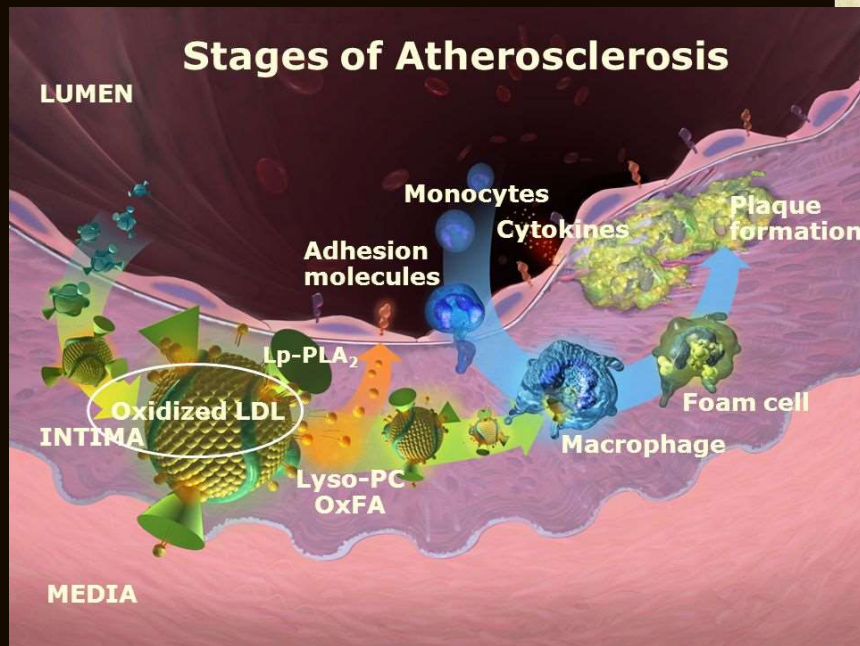




The EU regulation: a closer look

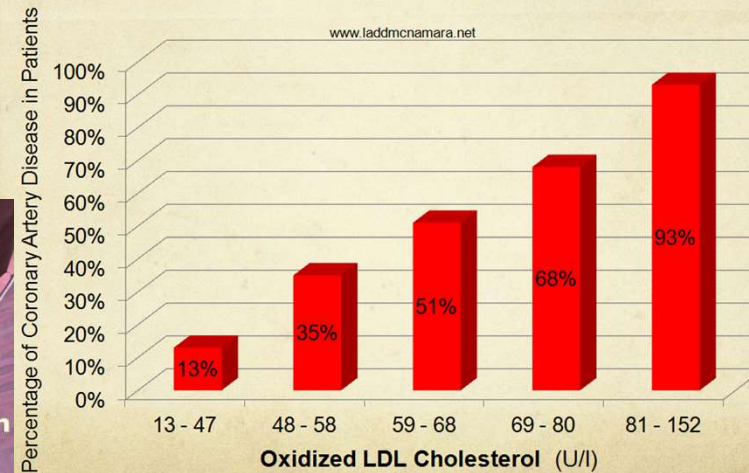
- 5 mg of hydroxytyrosol and derivatives (oleuropein complex and tyrosol) per day offer protection against LDL oxidation.
- Oleocanthal and oleacein are the two most abundant forms of conjugated hydroxytyrosol and tyrosol in most olive oils, together with oleuropein aglycon and ligstroside aglycon.

The significance of ox LDL

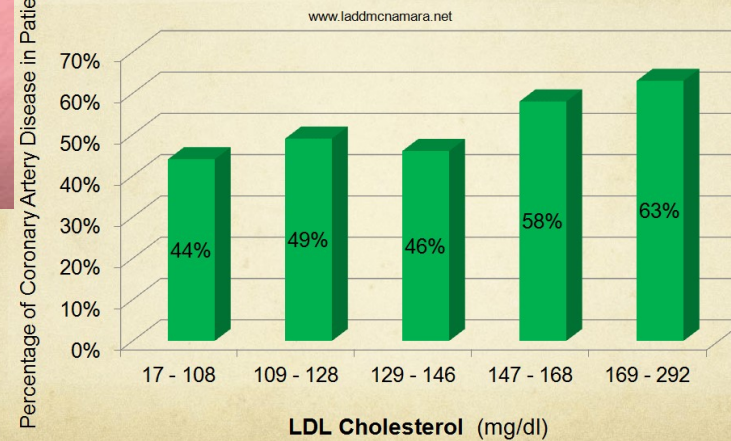


- The wording of the health claim is unfortunately not very "attractive"

Relationship Between **OXIDIZED** LDL Cholesterol and Coronary Artery Disease



Relationship Between LDL Cholesterol and Coronary Artery Disease



Recent experimental and new
clinical data

Beyond the EU health claim



Project co-financed by the European
Regional Development Fund

LDL/HDL-Total phenol content

EUROLIVE

n=200 (men) healthy

336 mg/kg vs 164 mg/kg vs 2.7 mg/kg; 25 ml/day

crossover; 3 weeks each oil

Linear increase in HDL

2.7 mg/kg = 0.9 mg/dl

164 mg/kg = 1.2 mg/dl

336 mg/kg = 1.7 mg/dl.

Compared to baseline levels:

366 mg/kg **decreased LDL** 6 mg/dl

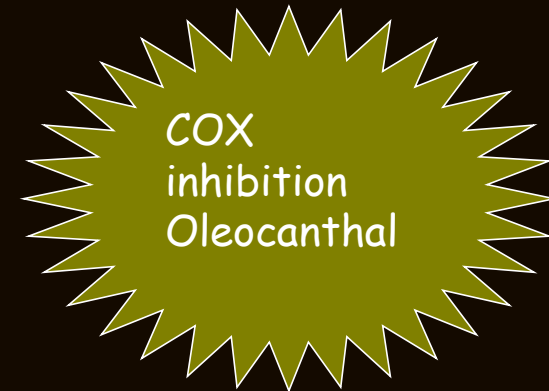
2.7 mg/dl increased LDL 6 mg/dl

Covas, MI. Ann Intern Med. 2006, 145:333

Castaner, O. AJCN. 2012, 95:1238

Three more human trials with unknown phenol content obtained either positive or neutral effects on HDL or LDL

Inflammation



- Spain

n = 24 (women)

564 mg/kg vs refined olive oil; 60 ml/day

crossover; 8 weeks each oil

Extra virgin olive oil with 564 mg/kg compared to baseline:

decreased c-reactive protein (CRP) 1.9 mg/L

Moreno-Luna, R. *Am J Hypertension*. 2012, 25:1299

- Spain

n= 28; stable coronary disease 161 mg/kg v 14.67 mg/kg; 50 ml

crossover; 3 weeks each oil

Extra virgin olive oil:


Fito, M. *Eur J Clin Nutr*. 2008, 62:570.

"the level of systemic inflammation as measured by circulating levels of C-reactive protein (CRP) has been linked to prognosis in patients with atherosclerotic disease, congestive heart failure, atrial fibrillation, myocarditis, aortic valve disease and heart transplantation."

RESEARCH ARTICLE



Effect of polyphenol-rich extra-virgin olive oil on lipid profile and inflammatory biomarkers in patients undergoing coronary angiography: a randomised, controlled, clinical trial

Nafiseh Khandouzi^a, Ali Zahedmehr^b and Javad Nasrollahzadeh^a 

^aDepartment of Clinical Nutrition & Dietetics, National Nutrition, and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran; ^bCardiovascular Intervention Research Center, Rajaie Cardiovascular, Medical & Research Center, Iran University of Medical Sciences, Tehran, Iran

ABSTRACT

The present study was conducted to compare the effects of high polyphenol extra-virgin olive oil (EVOO) with low polyphenol refined olive oil (ROO) on some cardiovascular risk factors in patients undergoing coronary angiography. In a randomised, controlled, parallel-arm, clinical trial, 40 patients with at least one classic cardiovascular risk factor who referred to coronary angiography were randomly allocated to two groups and received 25 mL EVOO or ROO daily for 6 weeks. Plasma LDL-cholesterol significantly reduced in EVOO group (-9.52 ± 20.44 vs 8.68 ± 18.77 mg/dL, $p = .007$ for EVOO and ROO respectively). EVOO resulted in a significant reduction in plasma CRP (-0.40 ± 0.52 vs 0.007 ± 0.42 mg/L, $p = .01$ for EVOO and ROO respectively) and increased ex-vivo whole blood LPS-stimulated IL-10 production (12.13 ± 33.64 vs -17.47 ± 49.04 pg/mL, $p = .035$ for EVOO and ROO respectively). Daily consumption of polyphenol-rich EVOO in subjects who have been under medical treatment with risk-reducing agents could additionally improve LDL-C and selected inflammatory markers.

Trial Registration Number: NCT03796780

ARTICLE HISTORY

Received 26 June 2020
Revised 20 October 2020
Accepted 20 October 2020

KEYWORDS

Extra-virgin olive oil; refined olive oil; lipid profile; inflammatory markers

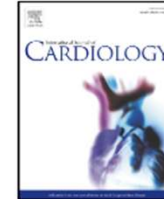
40 patients, 25 ml daily => reduction of cholesterol and inflammation markers



Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard



Post-prandial effects of high-polyphenolic extra virgin olive oil on endothelial function in adults at risk for type 2 diabetes: A randomized controlled crossover trial☆



Valentine Y. Njike^{a,b,*}, Rockiy Ayettey^{a,b}, Judith A. Treu^{a,b}, Kimberly N. Doughty^{a,b}, David L. Katz^a

^a Yale-Griffin Prevention Research Center, United States of America

^b Griffin Hospital, Derby, CT (VN, RA, JT, KD, DK), United States of America

- ✿ 20 patients with prediabetes
- ✿ Amelioration of endothelial function of blood vessels only with High phenolic olive oil in comparison to refined olive oil

Blood pressure / phenolic content

Spain

161 mg/kg vs refined; 50 ml/day; crossover; 3 weeks each oil

High phenolic EVOO: **lowered systolic BP**; no change in diastolic

Fito, M. Athero. 2005; 181:149.

Spain

564 mg/kg vs refined; 60 ml/day crossover; 8 weeks each oil

High phenolic EVOO: **lowered systolic and diastolic**

Moreno-Luna, R. Am J Hypertension. 2012, 25:1299

EUROLIVE

366 mg/kg vs 2.7 mg/kg; 25 ml/day; crossover; 3 weeks each oil

High phenolic EVOO: **decrease in diastolic BP** no change for systolic BP

Castaner, O. AJCN. 2012, 95:1238

Clinical studies for blood pressure in 2021








nutrients



Article

The Effect of High Polyphenol Extra Virgin Olive Oil on Blood Pressure and Arterial Stiffness in Healthy Australian Adults: A Randomized, Controlled, Cross-Over Study

Katerina Sarapis ¹, Colleen J. Thomas ², Johanna Hoskin ¹, Elena S. George ^{1,3} , Wolfgang Marx ^{1,4} , Hannah L. Mayr ^{5,6,7} , Greg Kennedy ⁸ , Andrew Pipingas ⁸, Jane C. Willcox ¹, Luke A. Prendergast ⁹, Catherine Itsiopoulos ^{5,10} and George Moschonis ^{1,*} 

50 patients with hypertension => drop 2.5 mm Hg of the blood pressure only with High Phenolic EVOO
In comparison with Low Phenolic EVOO
In 3 weeks

Effects of Extra Virgin Olive Oil Oleocanthal Content on Platelet Reactivity in Healthy Adults

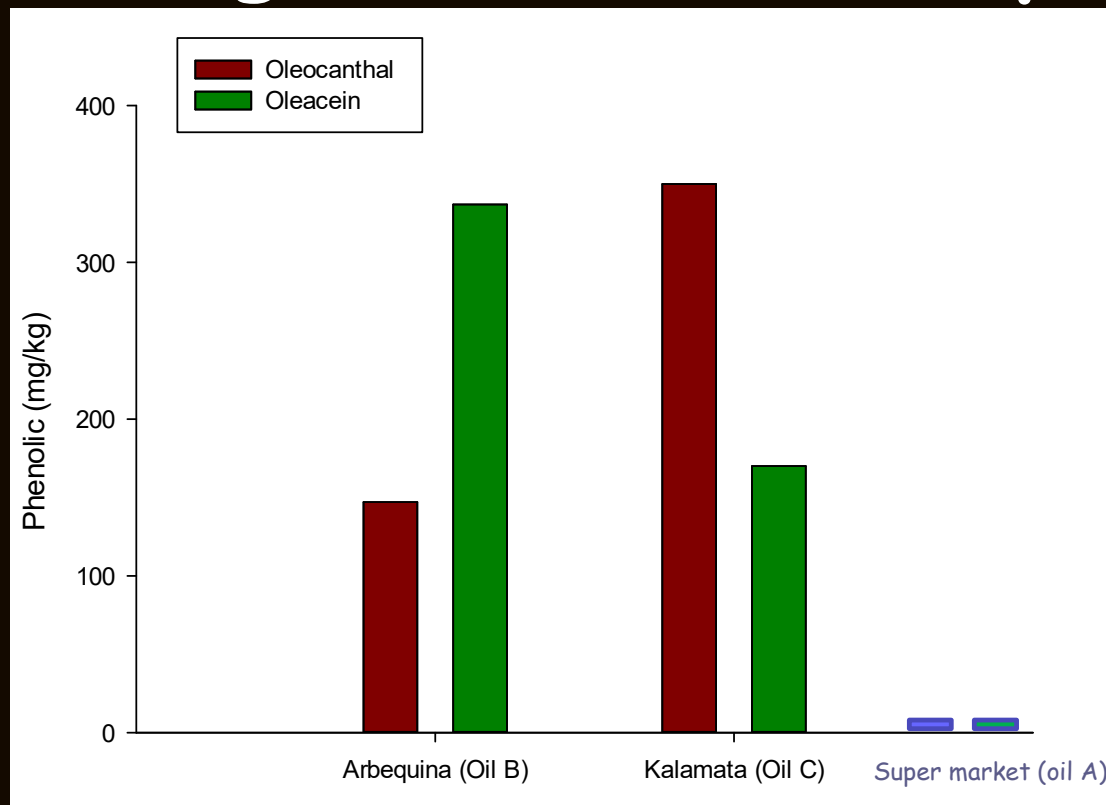


Department of
Nutrition



Western Human
Nutrition Research
Center

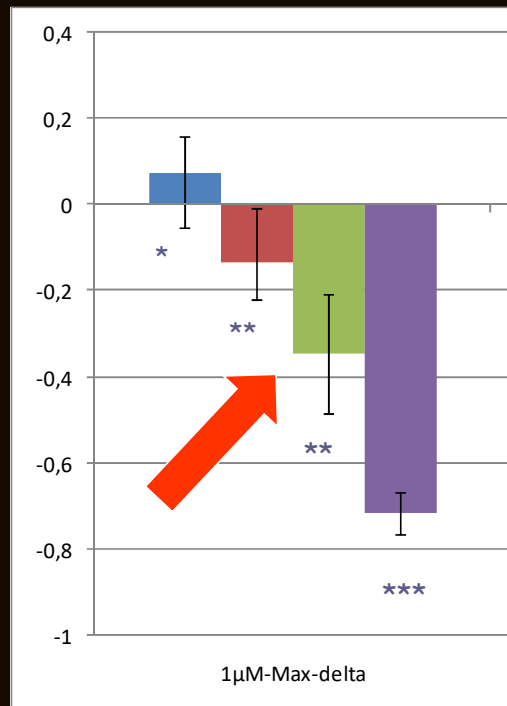
Extra Virgin Olive Oil Composition



Oil A: provided < 20 mg/kg oleocanthal; < 10 mg/kg oleacein

All oils provided similar amounts of total phenols (213 - 295 mg/kg caffeic acid equivalents) and had similar lipid profile

Oleocanthal and Oleacein-Rich EVOO Intake reduces Collagen-Induced Platelet Aggregation Relative to control EVOO



Blue: Super market control oil

Red: California Arbequina (High oleacein 12 mg dose)

Green: Kalamata oil (High oleocanthal 12 mg dose)

Purple: Ibuprofen (400 mg dose)

Collagen Stimulated
(n=9)



Oleocanthal and Alzheimer

Olive Oil Times Olive Oil World Reviews Health Business Making Olive Oil

How Extra Virgin Olive Oil Can Protect from Alzheimer's Disease

By ELENA PARAVANTES on March 26, 2013
Filed In Health | 1 Comment

1.3k SHARES

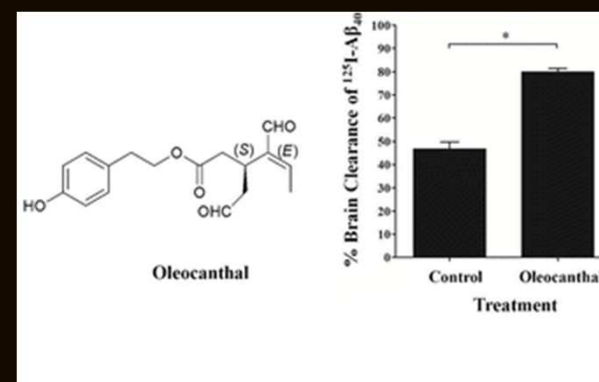


Olive oil has been associated with [protection against cognitive decline](#) that comes with aging, and studies have shown that the Mediterranean diet may reduce the risk of Alzheimer's disease and that the monounsaturated fat in olive oil was mainly responsible for this effect.

Now studies are now showing that it is in fact oleocanthal, a natural compound found in extra virgin olive oil that has antioxidant and anti-inflammatory action that may have the protective effect.

RELATED ARTICLES

Component in EVOO Kills Cancer Cells

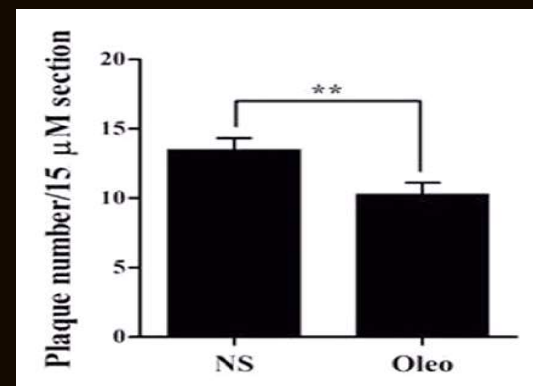
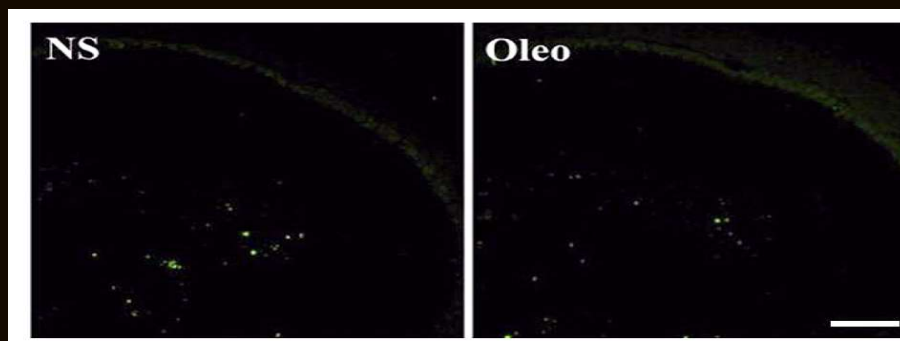
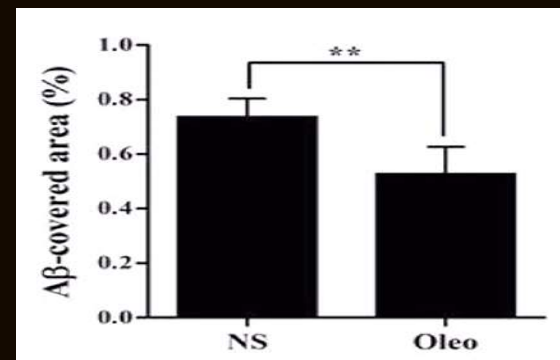
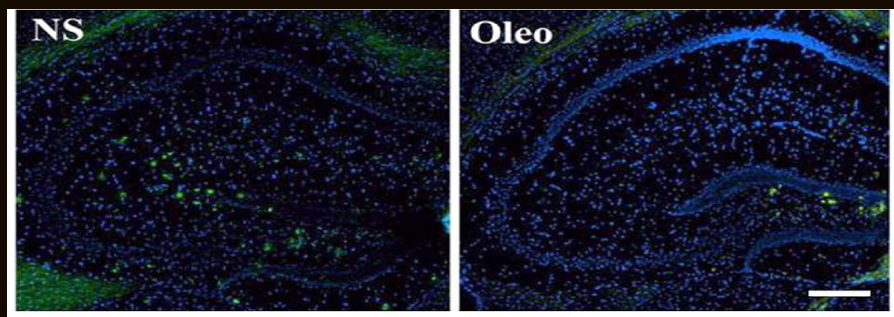


[ACS Chem Neurosci](#). 2013 Jun 19;4(6):973-82.

Olive-oil-derived oleocanthal enhances β -amyloid clearance as a potential neuroprotective mechanism against Alzheimer's disease: in vitro and in vivo studies.

[Abuznait AH](#), [Qosa H](#), [Busnena BA](#), [El Sayed KA](#), [Kaddoumi A](#).

Oleocanthal reduces total A β and A β plaques



4 weeks treatment i.p. starting at the age of 4 months reduces A β brain load in mice

Oleocanthal and Alzheimer



The Journal of Nutritional Biochemistry

Available online 27 December 2017

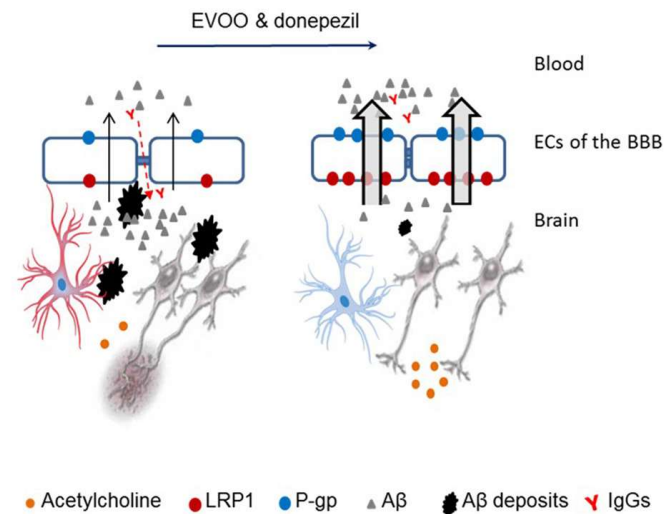
In Press, Accepted Manuscript ?



Oleocanthal-rich extra-virgin olive oil enhances donepezil effect by reducing amyloid- β load and related toxicity in a mouse model of Alzheimer's disease

Yazan S. Batarseh, Amal Kaddoumi ¹✉

Greek olive oil rich in oleocanthal has been able to stop the progression of the disease in experimental animals



Oleocanthal and Alzheimer

- Early harvest Olive oil Halkidiki rich in oleocanthal was able to reduce the symptoms and stop the progression of the disease in volunteers with mild cognitive dysfunction (early stage Alzheimer)
- The volunteers were consuming 40 ml of green olive oil per day for a year and they were compared with a second group of volunteers who were consuming the same amount of common olive oil
- The study was done in Thessaloniki by the Professor of Neurology Magda Tsolaki (Hellenic Society of Alzheimer)
- **CONFIRMATION** for the first time of the results that were previously observed in animals



Olive oil and Alzheimer

Journal of Alzheimer's Disease 78 (2020) 801–817
DOI 10.3233/JAD-200405
IOS Press

801

A Randomized Clinical Trial of Greek High Phenolic Early Harvest Extra Virgin Olive Oil in Mild Cognitive Impairment: The MICOIL Pilot Study

Magda Tsolaki^{a,b,*}, Eftychia Lazarou^b, Mahi Kozori^b, Niki Petridou^b, Irene Tabakis^a,
Ioulietta Lazarou^a, Maria Karakota^b, Iordanis Saoulidis^a, Eleni Melliou^c and Prokopios Magiatis^c
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^b*Greek Association of Alzheimer's Disease and Related Disorders, Thessaloniki, Makedonia, Greece*
^c*Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, National and
Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens, Greece*

Accepted 31 August 2020



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journal homepage: www.elsevier.com/locate/expgero



The pleiotropic beneficial intervention of olive oil intake on the Alzheimer's disease onset via fibrinolytic system

Elena E. Tzekaki^a, Magda Tsolaki^{b,c,**}, Anastasia A. Pantazaki^{a,*}, George Geromichalos^a,
Eftychia Lazarou^c, Mahi Kozori^c, Zacharias Sinakos^d

Experimental Gerontology 144 (2021) 111178



Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Restoration of BMI1 levels after the administration of early harvest extra virgin olive oil as a therapeutic strategy against Alzheimer's disease

Elena E. Tzekaki^{a,1}, Angelos Papaspyropoulos^{a,1}, Magda Tsolaki^{b,c,*}, Eftychia Lazarou^c,
Mahi Kozori^c, Anastasia A. Pantazaki^{a,**}

^a Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

^b 1st Department of Neurology, "AHEPA" General Hospital Medical School, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

^c Greek Association of Alzheimer's Disease and Related Disorders – GAARD, Greece

Olive oil and Multiple sclerosis



antioxidants



Article

Oleacein Attenuates the Pathogenesis of Experimental Autoimmune Encephalomyelitis through Both Antioxidant and Anti-Inflammatory Effects

Beatriz Gutiérrez-Miranda ¹, Isabel Gallardo ¹, Eleni Melliou ², Isabel Cabero ¹, Yolanda Álvarez ¹, Prokopios Magiatis ² , Marita Hernández ^{1,3,†} and María Luisa Nieto ^{1,*,†}

¹ Instituto de Biología y Genética Molecular (IBGM-CSIC/UVa), 47001 Valladolid, Spain; bgutimiranda@gmail.com (B.G.-M.); isa_gallard@hotmail.com (I.G.); netmabel@hotmail.com (I.C.); yolipi78@hotmail.com (Y.Á.); maritahg@ibgm.uva.es (M.H.)

² Laboratory of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, 15771 Athens, Greece; emelliou@pharm.uoa.gr (E.M.); magiatis@pharm.uoa.gr (P.M.)

³ Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad de Valladolid, 47001 Valladolid, Spain

* Correspondence: ml.nieto@csic.es; Tel.: +34-983-1848-36; Fax: +34-983-1848-00

† Equal contribution.

Received: 22 September 2020; Accepted: 18 November 2020; Published: 21 November 2020



Animal model: Experimental autoimmune encephalitis (EAE) induction

- 10 mg/kg/day, i.p injection

Figure 1: Clinical signs

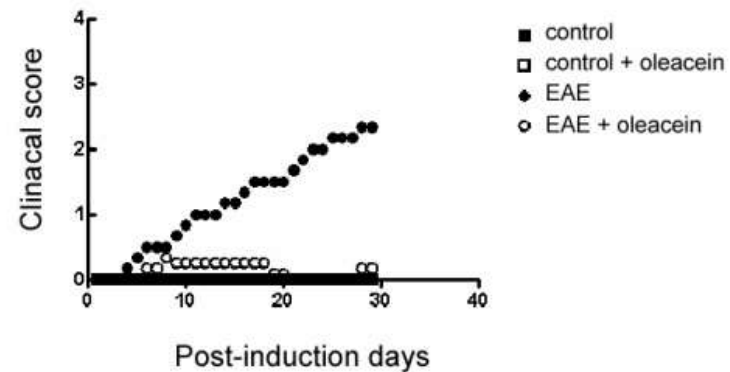
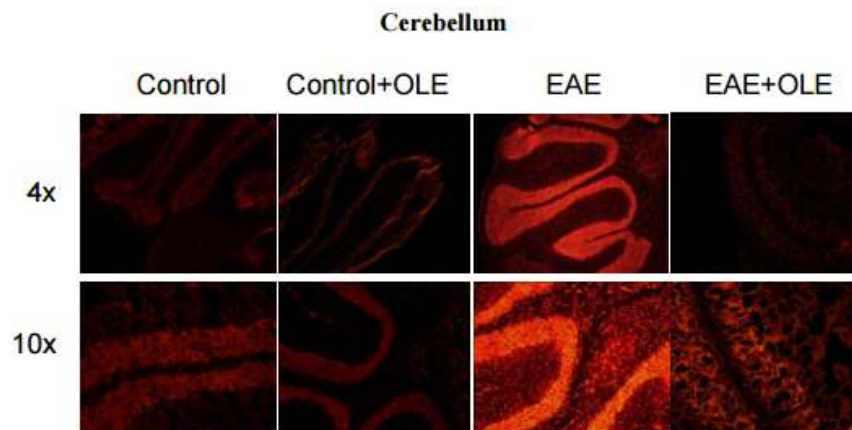


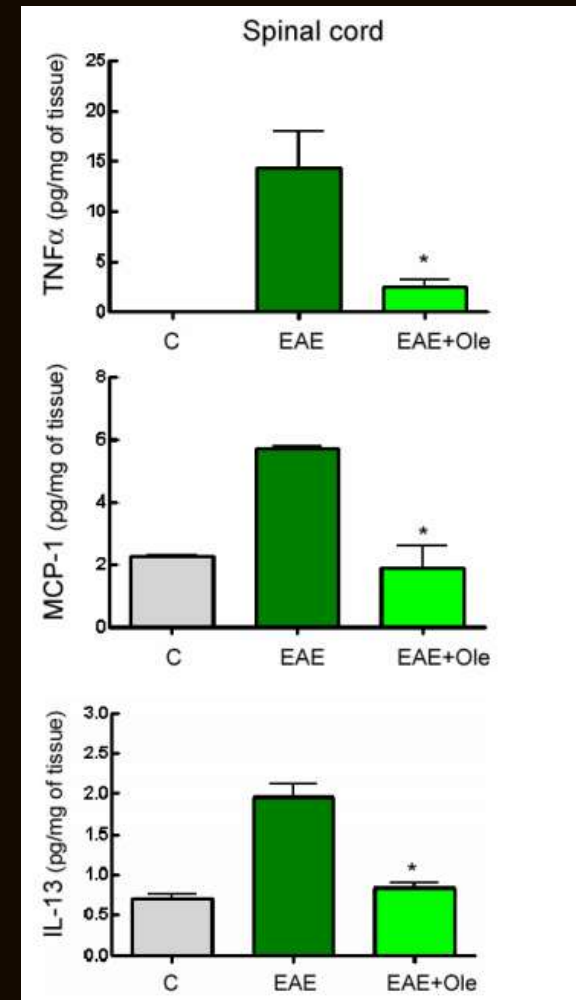
Figure 4: ROS production: DHE staining



In collaboration with:
Dr. Marisa Nieto
IBGM-CSIC/
Universidad de Valladolid
Spain

Animal model: Experimental autoimmune encephalitis (EAE) induction

- 10 mg/kg/day, i.p injection
- The olive oil phenols oleacein and oleocanthal can enter into the brain and exhibit a clear and strong protective activity
- Oleocanthal & Oleacein were isolated from E-LA-WON olive oil



Olive oil and multiple sclerosis Clinical study

ECRONICON
OPEN ACCESS

EC NEUROLOGY
Research Article

The Effect of Cretan High Phenolic Olive Oil on Fecal Calprotectin Levels in the Course of Multiple Sclerosis

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¹*Department of Psychology, University of Cyprus, Cyprus*

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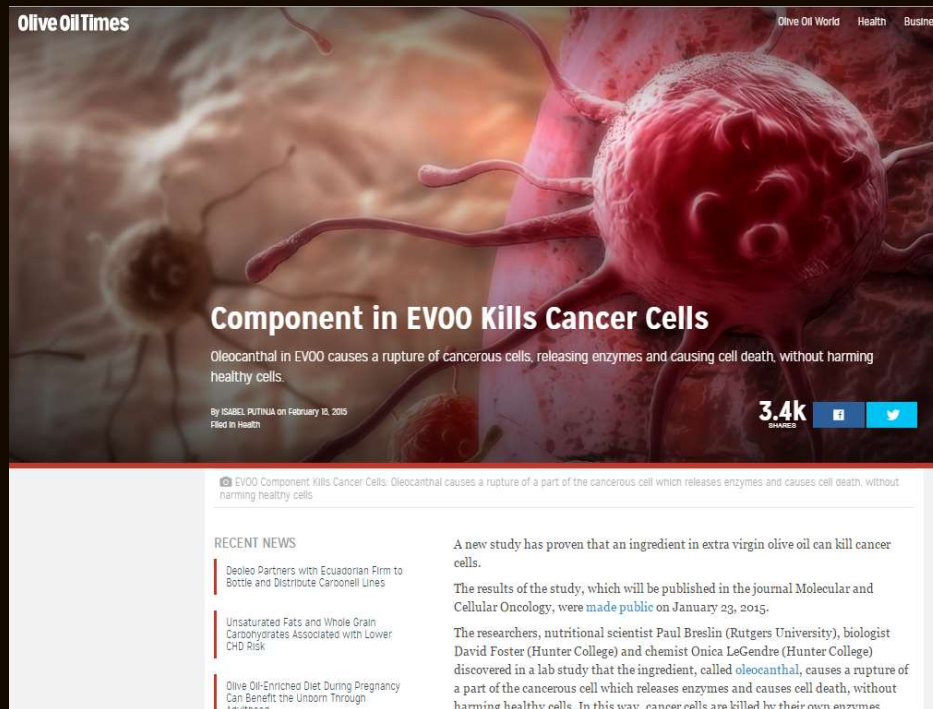
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✿ The study in Cyprus and Greece is in progress

Oleocanthal and cancer



- It has been found that Oleocanthal causes cancer cells to break down and die very quickly; within 30 minutes, instead of the 16 to 24 hours it takes for programmed apoptotic cell death.
- The proposed mechanism is that Oleocanthal disrupts the lysosome membrane
- Breslin P. et al Molecular & Cellular Oncology, 2015

Olive oil and Cancer*

Article

Effect of Dietary Intervention with High-Oleocanthal and Oleacein Olive Oil in Patients with Chronic Lymphocytic leukemia, a Pilot Randomized Trial

Andrea Paola Rojas Gil¹, Ioannis Kodonis², Anastasios Ioannidis¹, Tzortzis Nomikos³, Ioannis Dimopoulos⁴, Georgios Kosmidis¹, Maria Efthymia Katsa^{1,3}, Eleni Melliou⁵, Prokopios Magiatis^{5,†}

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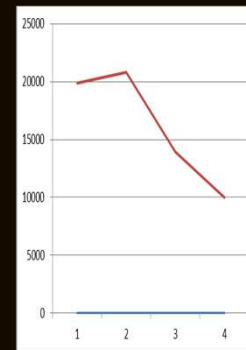
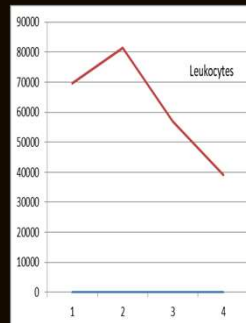
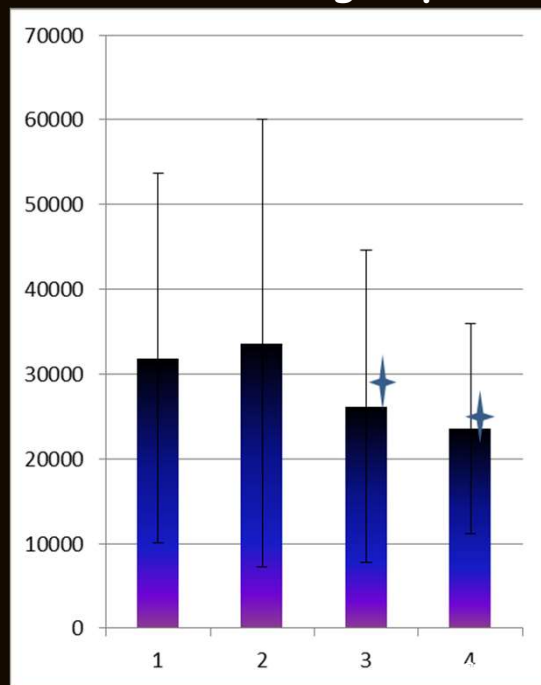
⁵Department of Pharmacy, Laboratory of Pharmacognosy and Natural Products Chemistry, National and Kapodistrian University of Athens, Greece

*submitted for publication

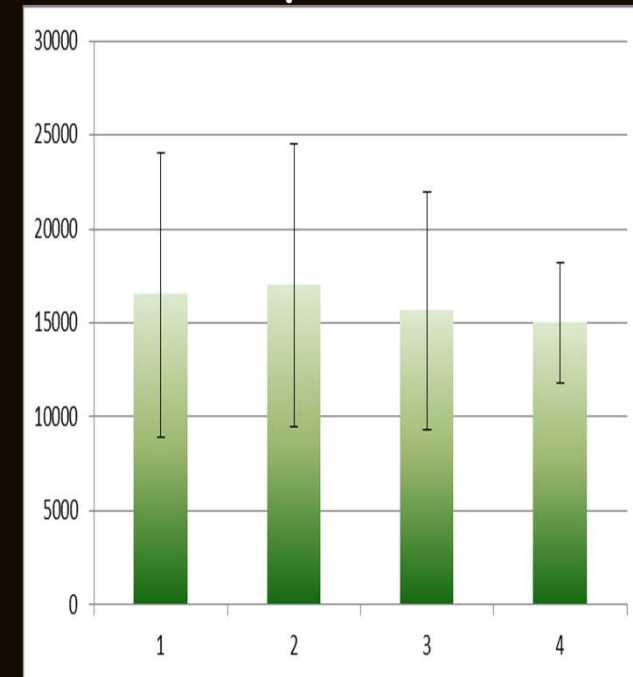
Results

Effect on white blood cells

WHITE BLOOD CELLS- LYMPHOCYTES
Intervention group



WHITE BLOOD CELLS
Placebo Group

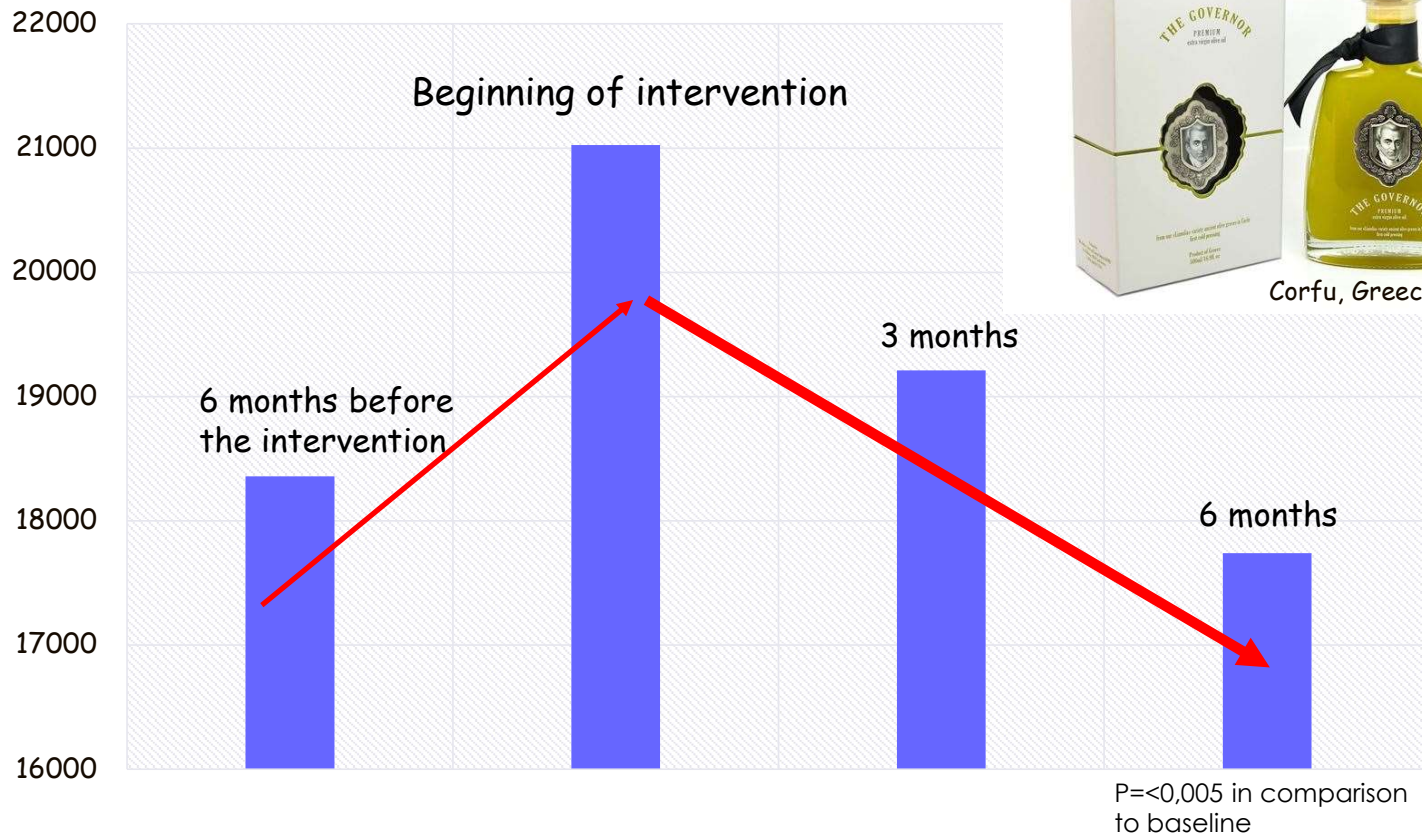


★ $P < 0.05$ $P < 0.05$ in relation to the baseline

A decrease in the number of white blood cells was observed in 22 patients of the intervention group after six months (40 ml per day)

Effect of high oleocanthal olive oil 40 ml/day for six months

White cells number in 22 CLL patients



The discovery of oleoglycine

Spontaneous *In Vitro* and *In Vivo* Interaction of (–)-Oleocanthal with Glycine in Biological Fluids: Novel Pharmacokinetic Markers

Lucy I. Darakjian,[#] Aimilia Rigakou,[#] Andrew Brannen, Mohammed H. Qusa, Niki Tasiakou, Panagiotis Diamantakos, Miranda N. Reed, Peter Panizzi, Melissa D. Boersma, Eleni Melliou, Khalid A. El Sayed, Prokopios Magiatis,^{*} and Amal Kaddoumi^{*}



Cite This: <https://dx.doi.org/10.1021/acsptsci.0c00166>



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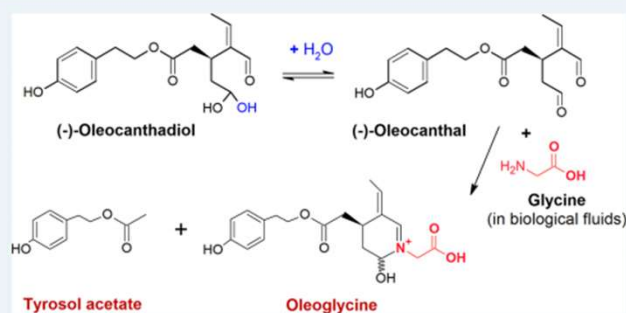
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Metrics & More

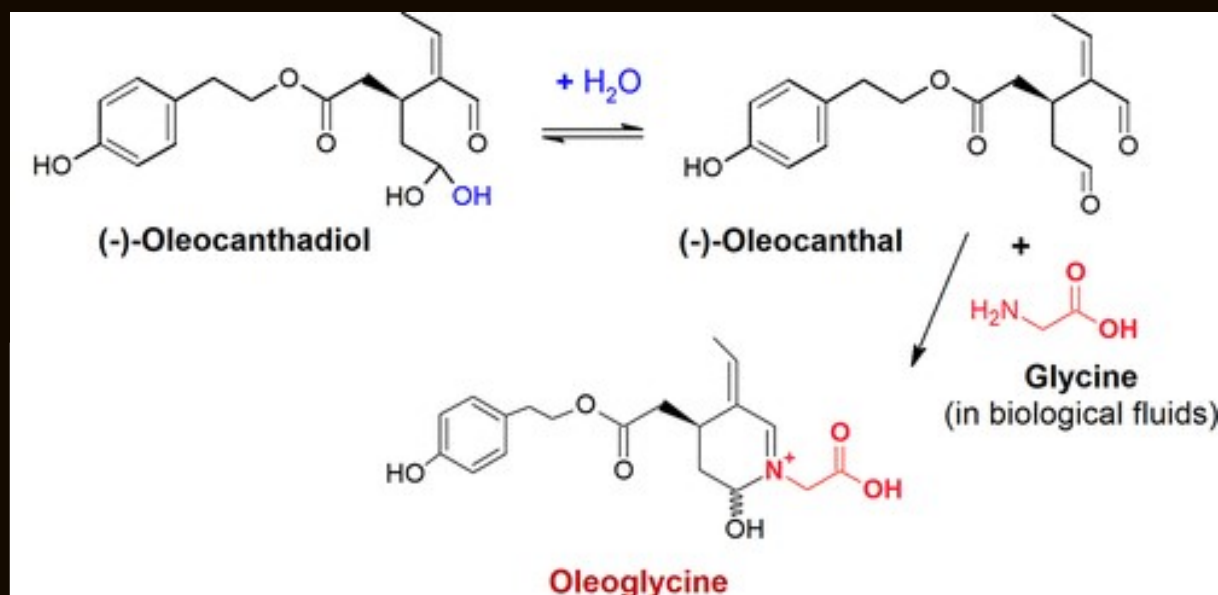
Article Recommendations

Supporting Information

ABSTRACT: Since the first discovery of its ibuprofen-like anti-inflammatory activity in 2005, the olive phenolic (–)-oleocanthal gained great scientific interest and popularity due to its reported health benefits. (–)-Oleocanthal is a monophenolic secoiridoid exclusively occurring in extra-virgin olive oil (EVOO). While several groups have investigated oleocanthal pharmacokinetics (PK) and disposition, none was able to detect oleocanthal in biological fluids or identify its PK profile that is essential for translational research studies. Besides, oleocanthal could not be detected following its addition to any fluid containing amino acids or proteins such as plasma or culture media, which could be attributed to its unique structure with two highly reactive aldehyde



How oleocanthal circulates in the blood



- It reacts spontaneously with glycine that is present in every biological fluid and is transformed into oleoglycine that crosses the blood-brain barrier and reaches the brain where it exerts its action.
- ACS Pharmacology 2021, 4, 1, 179–192

Clinical studies in progress

- Today five clinical trials with specially selected olive oils are in progress for:
 - Chronic Lymphocytic Leukemia-Multiple myeloma
 - Alzheimer
 - Multiple sclerosis
 - Platelet aggregation
 - Diabetes
- Not generally rich in polyphenols but rich in specific polyphenols (oleocanthal or oleacein) and in particular the oleocanthal that seems to characterize most Greek varieties.
- The studied oils are precisely characterized in the same way with the officially recognized herbal medicines.

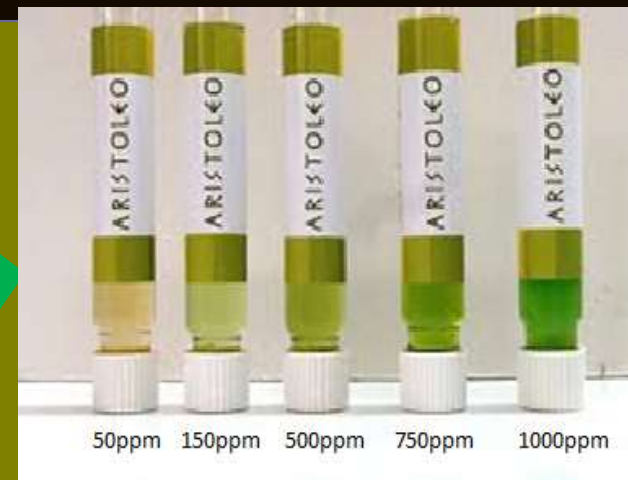
TARGETS

- Our ultimate target is to collect sufficient clinical data to transform the high-phenolic or high oleocanthal olive oils from healthy foods and food supplements to medical foods or herbal medicines
- To demonstrate the effectiveness and safety of the pure active ingredients like oleocanthal in humans and provide real new drugs coming from olive oil

Need for certified EVOOs with polyphenol health claim

- Organoleptic evaluation is not enough
- The health claim can be certified by precise measurement of all the phenols included in the health claim regulation using expensive laboratory methods like the qNMR
- To make things easier we invented a new colorimetric reaction "the ARISTOLEO test" that can offer direct identification of the high phenolic EVOOs
- The ARISTOLEO test can give quantitative measurement of the oleocanthal and oleacein content with the ARISTOMETRO device

ARISTOLEO test kit



ARISTOMETRO

- Automatization of colorimetric measurement in 20 min



Demonstration



THANKS TO

- Eleni Melliou
- Panagiotis Diamantakos, Annia Tsolakou, Christos Papanikolaou, Aimilia Rigakou, Katerina Papakonstantinou, (Univ. of Athens)
- The partners of the ARISTOIL project



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