"Olive Oil Polyphenols Contribute to the Protection of Blood Lipids from Oxidative Stress" AND THE FATE OF EC REG. 432/2012: ARE THE BASIC TERMS AND THE FIGURES ANY CLEARER NOW?

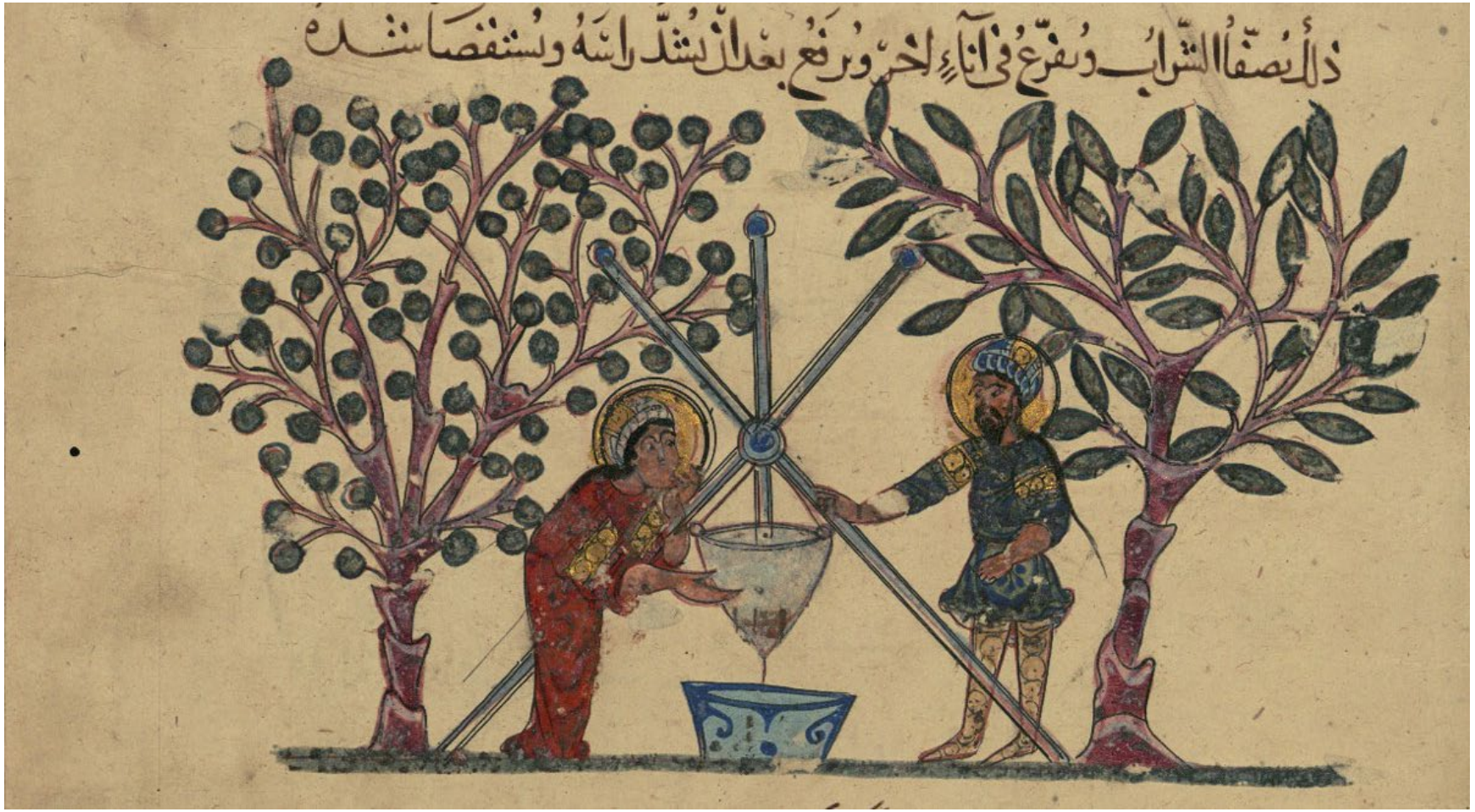
"Olive Oil Polyphenols Contribute to the Protection of Blood Lipids from Oxidative Stress" AND THE FATE OF EC REG. 432/2012: ARE THE BASIC TERMS AND THE FIGURES ANY CLEARER NOW?

"Olive Oil Polyphenols Contribute to the Protection of Blood Lipids from Oxidative Stress" AND THE FATE OF EC REG. 432/2012: ARE THE BASIC TERMS AND THE FIGURES ANY CLEARER NOW

Bojan BUTINAR
Gašper KOZLOVIČ
Milena BUČAR-MIKLAVČIČ

Science and Research Centre Koper
Laboratory of the Institute of the Oliveculture
Izola, Slovenia

PEDANIUS DIOSCORIDES (De Materia Medica) 40-90 AD



MOTTO 1

There‘s been rumors of war and wars that have been
The meaning of life has been lost in the wind
And some people thinkin‘ that the end is close by
S‘tead of learnin‘ to live they are learning to die.
Let me die in my footsteps
Before I go down under the ground.

- Bob Dylan – Let Me Die in My Footsteps (1963)

MOTTO 2

The philosophers have only *interpreted* the world, in various ways; **the point, however, is to *change* it.**

- Karl Marx – Theses on Feuerbach, XI (tr. from 1888 ed.)

BIOPHENOLS (BP)

HC EU 432/2012 [EC, 2012]

Relevant entry number: 1333, 1368, 1639, 1696, 2865

“Olive Oil Polyphenols Contribute to the Protection of Blood Lipids from Oxidative Stress”

Conditions of use of the Claim

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".

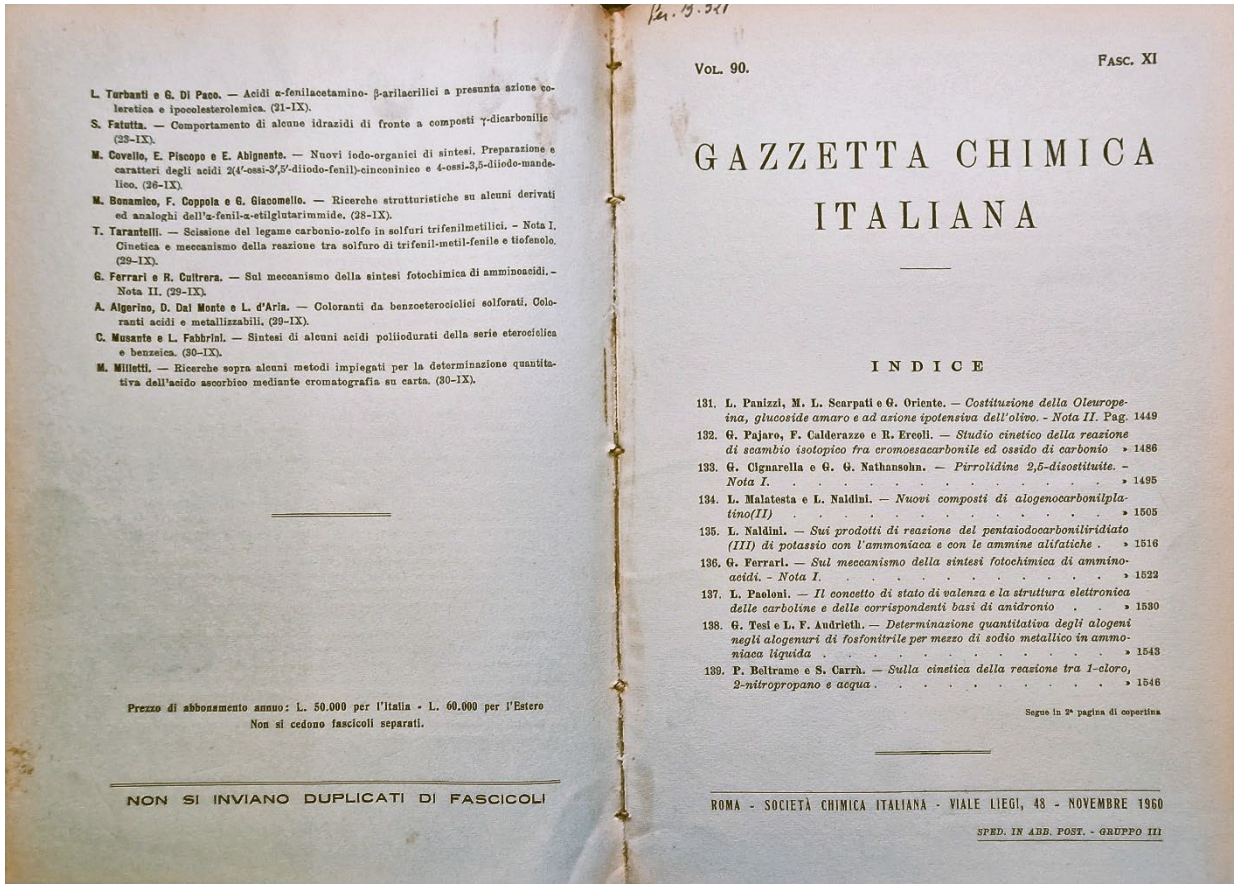
OPUS PRÆTERITUM

Why do we think we can comment on the BP riddle?

- **Biophenols** research 1995 –
- In the validation ring for IOC method ***Determination of biophenols*** (2007)
- **Accredited** according to ISO 17025
- GREX IOC from 2001 – sensorics & chemistry
- GREX EU from 2006
- Horizont 2020 OLEUM Project: Sensorics & BP & TAG composition
- Work with prof. dr. **Maria Tsimidou** & dr. **Nikolaos Nenadis**(AUTH, BP Oleum)

WHO IS GUILTY?

- Hommage to Luigi Panizzi [PANIZZI et al., 1960]



L. Turbanti e G. Di Paco. — Acidi α-fenilacetamino- β-arilacrilici a presunta azione coleretica e ipocolesterolemica. (21-IX).
S. Fatutta. — Comportamento di alcune idrazidi di fronte a composti γ-dicarbonilici (23-IX).
M. Covello, E. Piscopo e E. Abignente. — Nuovi iodo-organici di sintesi. Preparazione e caratteri degli acidi 2(4'-ossi-3',5'-diiodo-fenil)-cinconinico e 4-ossi-3,5-diiodo-mandelico. (26-IX).
M. Bonamico, F. Coppola e G. Giacomello. — Ricerche strutturistiche su alcuni derivati ed analoghi dell'α-fenil-α-etilglutarimmide. (28-IX).
T. Tarantelli. — Scissione del legame carbonio-zolfo in solfuri trifenilmetilici. – Nota I. Cinetica e meccanismo della reazione tra solfuro di trifenil-metil-fenile e tiofenolo. (29-IX).
G. Ferrari e R. Cuttrera. — Sul meccanismo della sintesi fotochimica di amminoacidi. – Nota II. (29-IX).
A. Algerino, D. Dal Monte e L. d'Aria. — Coloranti da benzoeterocicilici solforati. Coloranti acidi e metallizzabili. (29-IX).
C. Musante e L. Fabbrini. — Sintesi di alcuni acidi poliiodurati della serie eterociclica e benzeica. (30-IX).
M. Milletti. — Ricerche sopra alcuni metodi impiegati per la determinazione quantitativa dell'acido ascorbico mediante cromatografia su carta. (30-IX).

Prezzo di abbonamento annuo: L. 50.000 per l'Italia - L. 60.000 per l'Estero
Non si cedono fascicoli separati.

NON SI INVIANO DUPLICATI DI FASCICOLI

VOL. 90. FASC. XI

GAZZETTA CHIMICA ITALIANA

INDICE

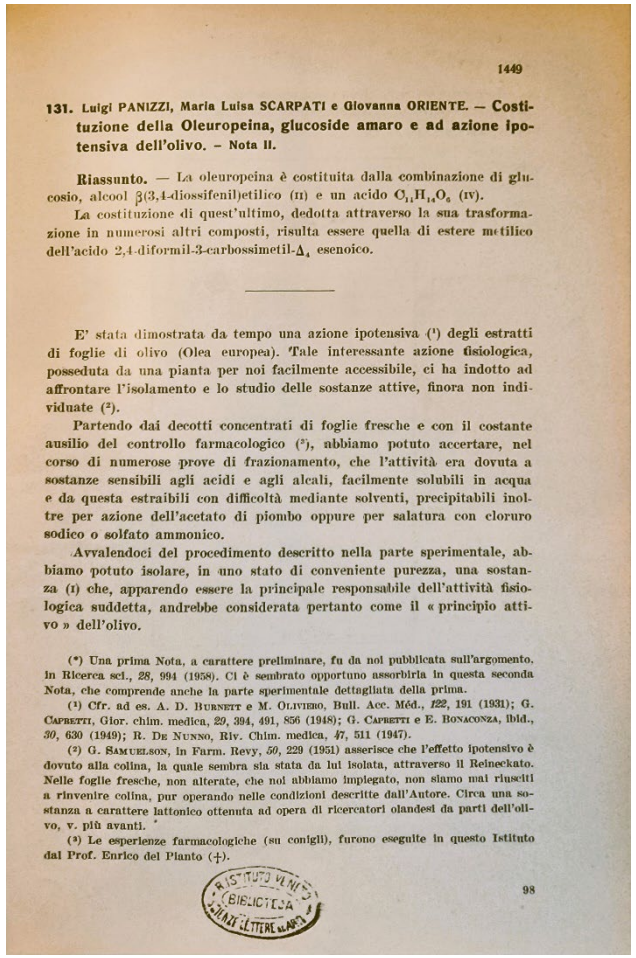
Segue in 2ª pagina di copertina

ROMA - SOCIETÀ CHIMICA ITALIANA - VIALE LIEGI, 48 - NOVEMBRE 1960

SPED. IN ABB. POST. - GRUPPO III

WHO IS GUILTY?

Costituzione della Oleuropeina, glucoside amaro e ad azione ipotensiva dell‘olivo



1449

131. Luigi PANIZZI, Maria Luisa SCARPATI e Giovanna ORIENTE. — Costituzione della Oleuropeina, glucoside amaro e ad azione ipotensiva dell'olivo. – Nota II.

Riassunto. — La oleuropeina è costituita dalla combinazione di glucosio, alcool β(3,4-diossifenil)etilico (II) e un acido $C_{11}H_{12}O_6$ (IV).

La costituzione di quest'ultimo, dedotta attraverso la sua trasformazione in numerosi altri composti, risulta essere quella di estere metilico dell'acido 2,4-diformil-3-carbossimetil-Δ_4 esenoico.

E' stata dimostrata da tempo una azione ipotensiva (1) degli estratti di foglie di olivo (Olea europea). Tale interessante azione fisiologica, posseduta da una pianta per noi facilmente accessibile, ci ha indotto ad affrontare l'isolamento e lo studio delle sostanze attive, finora non individuate (2).

Partendo dai decotti concentrati di foglie fresche e con il costante ausilio del controllo farmacologico (3), abbiamo potuto accertare, nel corso di numerose prove di frazionamento, che l'attività era dovuta a sostanze sensibili agli acidi e agli alcali, facilmente solubili in acqua e da questa estraibili con difficoltà mediante solventi, precipitabili inoltre per azione dell'acetato di piombo oppure per salatura con cloruro sodico o solfato ammonico.

Avvalendoci del procedimento descritto nella parte sperimentale, abbiamo potuto isolare, in uno stato di conveniente purezza, una sostanza (I) che, apparendo essere la principale responsabile dell'attività fisiologica suddetta, andrebbe considerata pertanto come il « principio attivo » dell'olivo.

(*) Una prima Nota, a carattere preliminare, fu da noi pubblicata sull'argomento, in Ricerca sci., *28*, 994 (1958). Ci è sembrato opportuno assorbirla in questa seconda Nota, che comprende anche la parte sperimentale dettagliata della prima.

(1) Cfr. ad es. A. D. Burnett e M. Oliviero, Bull. Acc. Méd., *122*, 191 (1931); G. Capretti, Gior. chim. medica, *29*, 394, 491, 856 (1948); G. Capretti e E. Bonaconza, ibid., *30*, 630 (1949); R. De Nunno, Riv. Chim. medica, *47*, 511 (1947).

(2) G. Samuelson, in Farm. Revy, *50*, 229 (1951) asserisce che l'effetto ipotensivo è dovuto alla colina, la quale sembra sia stata da lui isolata, attraverso il Reineckato. Nelle foglie fresche, non alterate, che noi abbiamo impiegato, non siamo mai riusciti a rinvenire colina, pur operando nelle condizioni descritte dall'Autore. Circa una sostanza a carattere lattonico ottenuta ad opera di ricercatori olandesi da parti dell'olivo, v. più avanti.

(3) Le esperienze farmacologiche (su conigli), furono eseguite in questo Istituto dal Prof. Enrico del Pianto (†).

98

WHAT ARE BP IN (E)VOO?

- They are ***aromatic compounds*** with one or more hydroxyl groups. Phenol substances in wine area and their research caused the term polyphenols to spread to olives and (unfortunately) remained there. Just like *Indians* or *Russian tea* ...
- In olive tree and in the family *Oleaceae*, BPs are ***secoiridoids*** of monoterpenoid origin, linked to a glucose unit (OLE, LIG).
- They are mainly represented in the olive *mesocarp* and the *leaves*...

Why are they there? Above all, their function is defensive. *Oleuropein* releases phytoalexins and thus protects the olive.

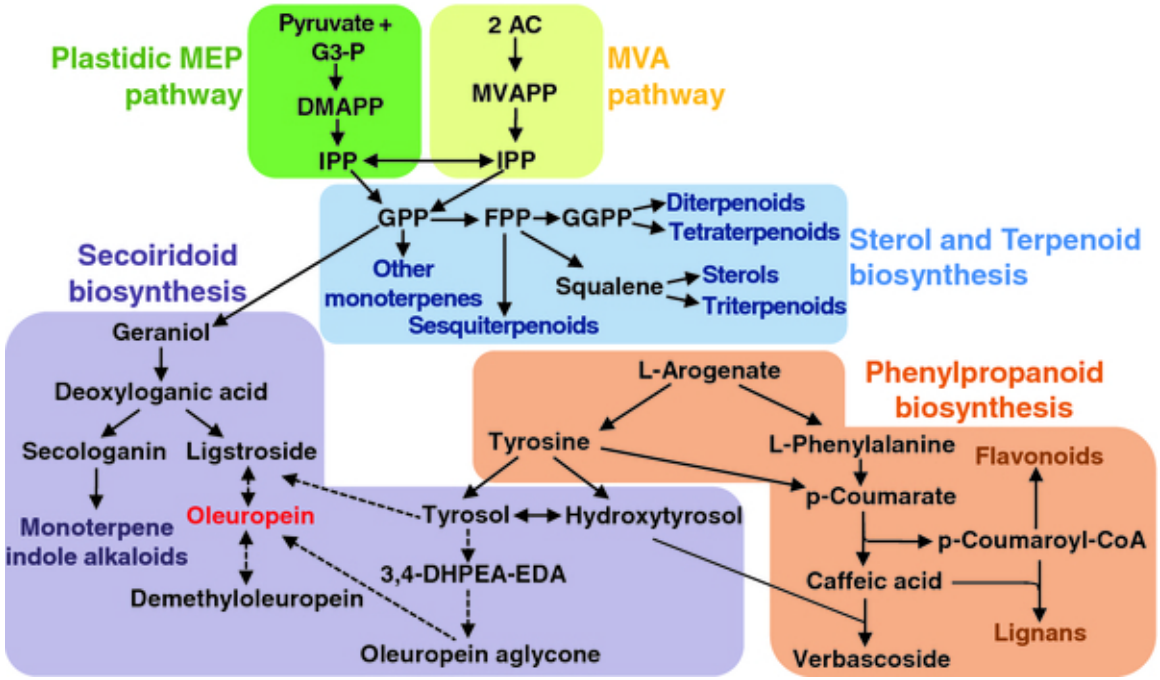
[Alagna et al., 2012]

BIOPHENOLS

Biogenesis of BP?

- From mevalonic acid pathway (MVAP) thru geranyl diphosphate (GPP)
- Intra conversions – Lig – OLE and their derivatives!

[Alagna et al., 2012]



BP ARE MORE THAN ONE!

27 HPLC separable molecules: 1P-1M

Red ones are secoiridoids

Hydroxytyrosol

Tyrosol

Vanillic acid

Caffeic acid

Vanillin

Para-coumaric acid

Hydroxytyrosyl acetate

Ferulic acid

Ortho-coumaric acid

Decarboxymethyl oleuropein aglycone, oxidised dialdehyde form

Decarboxymethyl oleuropein aglycone, dialdehyde form

Oleuropein

Oleuropein aglycone, dialdehyde form

Tyrosyl acetate

Decarboxymethyl ligstroside aglycone, oxidised dialdehyde form

Decarboxymethyl ligstroside aglycone, dialdehyde form

Pinoresinol

1-acetoxy-pinoresinol

Cinnamic acid

Ligstroside aglycone, dialdehyde form

Oleuropein aglycone, oxidised aldehyde and hydroxylic form

Luteolin

Oleuropein aglycone, aldehyde and hydroxylic form

Ligstroside aglycone, oxidised aldehyde and hydroxylic form

Apigenin

Methyl-luteolin

Ligstroside aglycone, aldehyde and hydroxylic form

[CONTE, 2022]

BIOPHENOLS

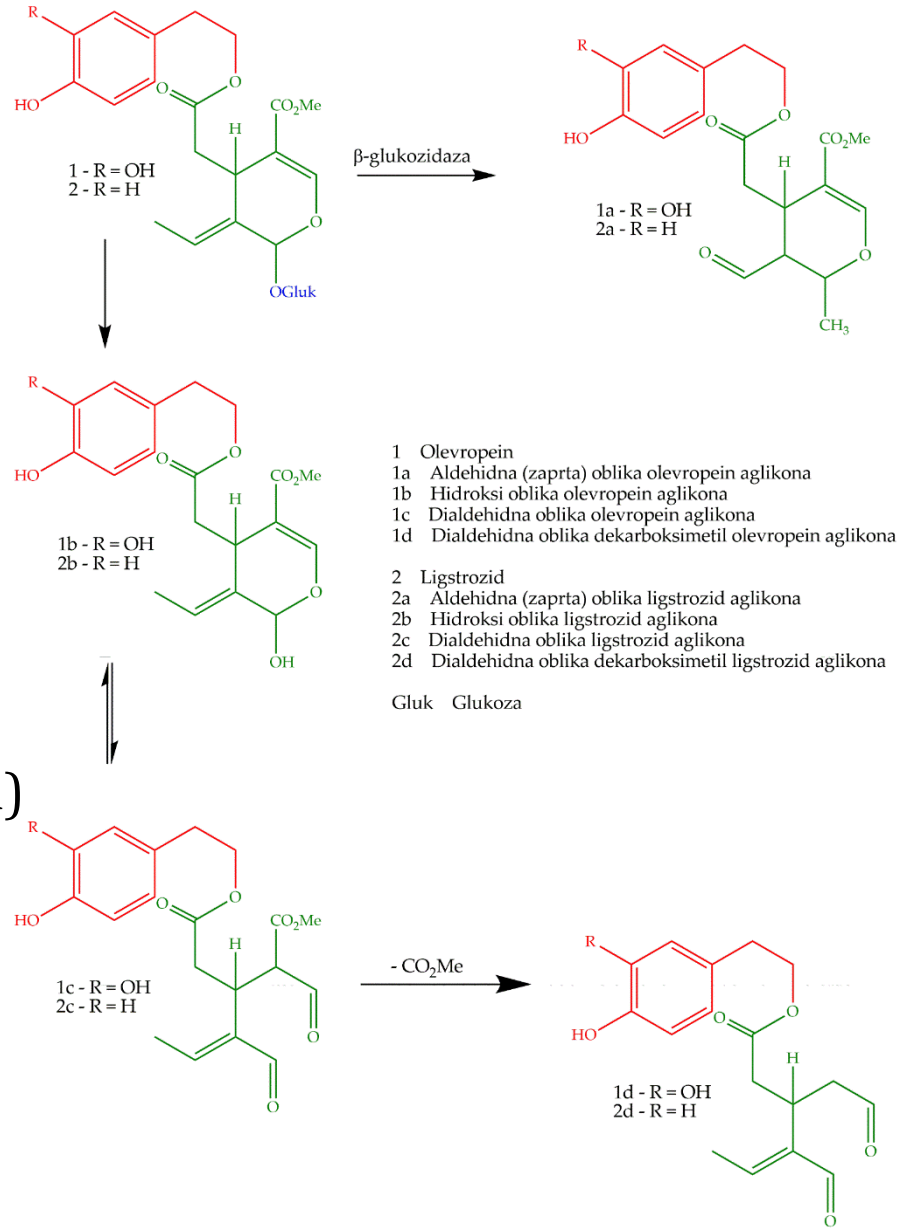
2 Main: LIG & OLE

- Derivatives
- Or (Conversion products)

ARE SHOWN

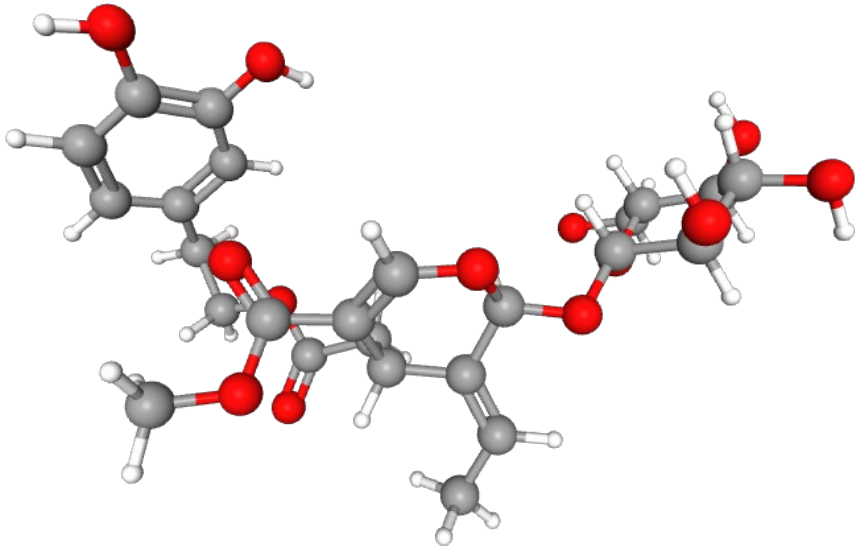
The final ones (not shown, in red) are TyrOH and Tyr.

[PODGORNIK at al., 2021]



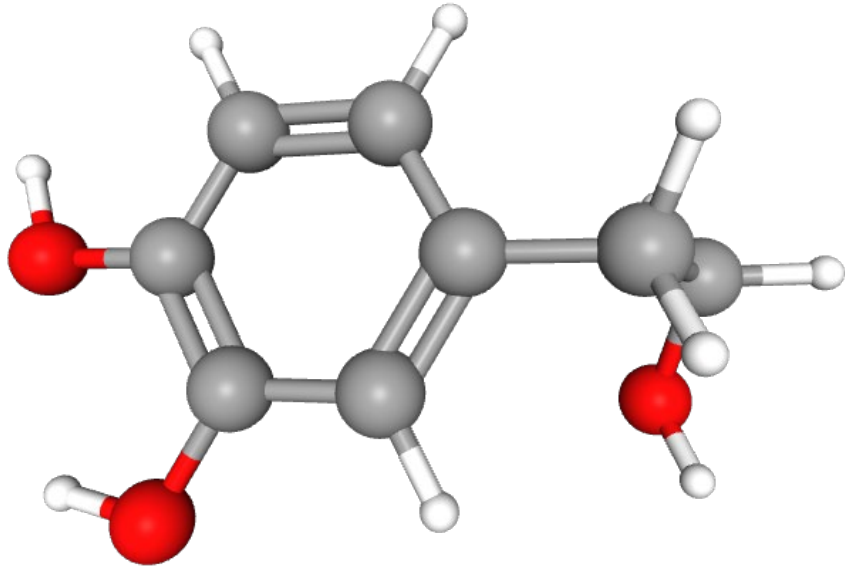
BP – formulas

A. OLE and LIG
B. TyrOH + Tyr
C. Oleacein + Oleocanthal
D. OLE-Agly + LIG-Agly
E. Oleomissional + Oleocoronal
F. Oleuropeindial + Ligstrodial



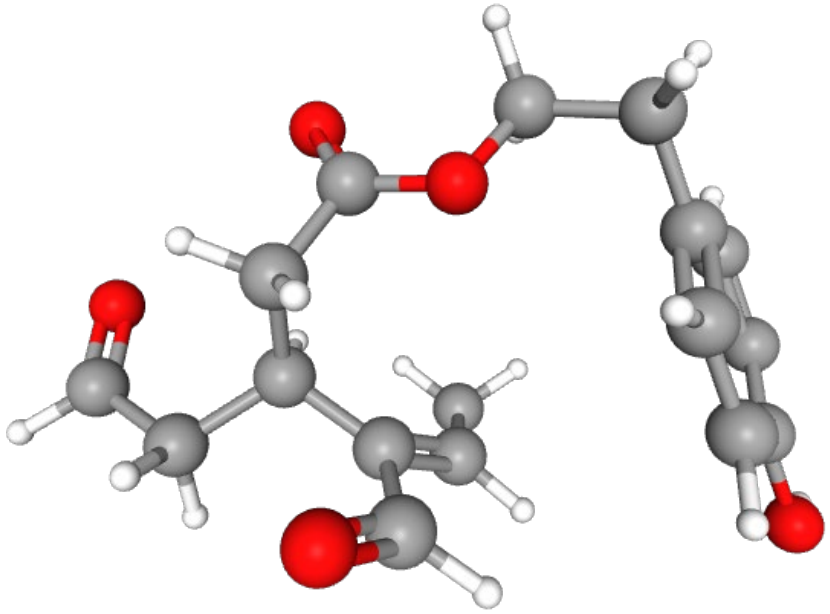
BP – formulas

A. OLE and LIG
B. TyrOH + Tyr
C. Oleacein + Oleocanthal
D. OLE-Agly + LIG-Agly
E. Oleomissional + Oleocoronal
F. Oleuropeindial + Ligstrodial



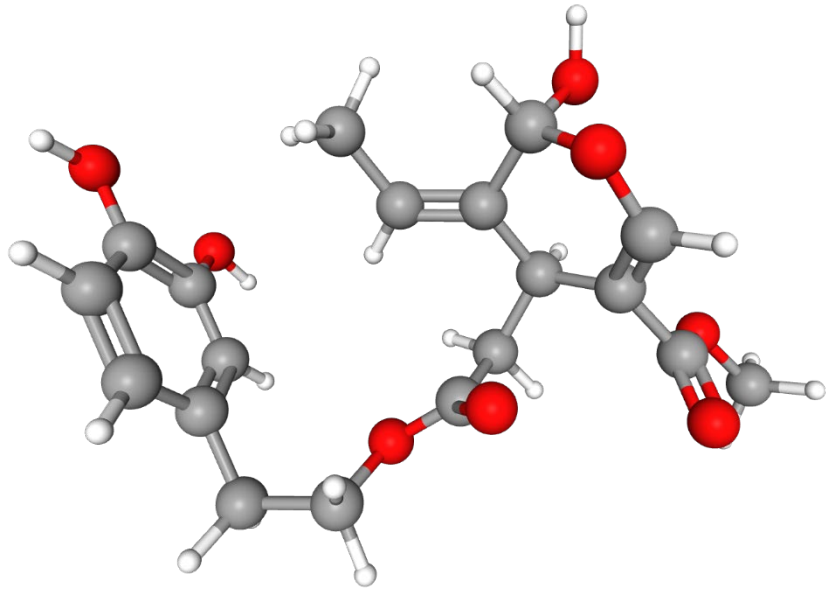
BP – formulas

A. OLE and LIG
B. TyrOH + Tyr
C. Oleacein + Oleocanthal
D. OLE-Agly + LIG-Agly
E. Oleomissional + Oleocoronal
F. Oleuropeindial + Ligstrodial



BP – formulas

A. OLE and LIG
B. TyrOH + Tyr
C. Oleacein + Oleocanthal
D. OLE-Agly + LIG-Agly
E. Oleomissional + Oleocoronal
F. Oleuropeindial + Ligstrodial



BIOPHENOLS (BP)

HC EU 432/2012 [EC, 2012]

Relevant entry number: 1333, 1368, 1639, 1696, 2865

“Olive Oil Polyphenols Contribute to the Protection of Blood Lipids from Oxidative Stress”

Conditions of use of the Claim

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.

BIOPHENOLS (BP)

HC EU 432/2012 [EC, 2012]

Relevant entry number: 1333, 1368, 1639, 1696, 2865

“Olive Oil Polyphenols Contribute to the Protection of Blood Lipids from Oxidative Stress”

And Pandora‘s Box was opened!

Conditions of use of the Claim

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.

EFSA SCIENTIFIC OPINION BASIS

- EFSA‘s SO [SCIENTIFIC…, 2011] was based on 16 references
- Mostly medical substantiation…
- With analytical details only 4 (in red)
- Only 2 among them [DE LA TORRE-CARBOT et al., 2010] & [WEINBRENNER et al., 2004] with AMD
- Only ONE relevant (EVOO!) with cited references with correct and exact, and useful BP terminology

EFSA SCIENTIFIC OPINION LITERATURE BASIS

Bonanome A, Pagnan A, Caruso D, Toia A, Xamin A, Fedeli E, Berra B, Zamburlini A, Ursini F and Galli G, 2000. Evidence of postprandial absorption of olive oil phenols in humans. *Nutrition, Metabolism and Cardiovascular Diseases*, **10**, 111– 120.

Covas MI, de la Torre K, Farre-Albaladejo M, Kaikkonen J, Fito M, Lopez-Sabater C, Pujadas-Bastardes MA, Joglar J, Weinbrenner T, Lamuela-Raventos RM and de la Torre R, 2006a. Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. *Free Radical Biology and Medicine*, 40, 608– 616.

Covas MI, Nyyssonen K, Poulsen HE, Kaikkonen J, Zunft HJ, Kiesewetter H, Gaddi A, de la Torre R, Mursu J, Baumler H, Nascetti S, Salonen JT, Fito M, Virtanen J, Marrugat J and Group ES, 2006b. The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. *Annals of Internal Medicine*, 145, 333– 341.

de la Torre-Carbot K, Chavez-Servin JL, Jauregui O, Castellote AI, Lamuela-Raventos RM, Nurmi T, Poulsen HE, Gaddi AV, Kaikkonen J, Zunft HF, Kiesewetter H, Fito M, Covas MI and Lopez-Sabater MC, 2010. Elevated circulating LDL phenol levels in men who consumed virgin rather than refined olive oil are associated with less oxidation of plasma LDL. *Journal of Nutrition*, 140, 501– 508.

Edgecombe SC, Stretch GL and Hayball PJ, 2000. Oleuropein, an antioxidant polyphenol from olive oil, is poorly absorbed from isolated perfused rat intestine. *Journal of Nutrition*, **130**, 2996– 3002.

Gilani AH, Khan AU, Shah AJ, Connor J and Jabeen Q, 2005. Blood pressure lowering effect of olive is mediated through calcium channel blockade. *International Journal of Food Sciences and Nutrition*, **56**, 613– 620.

Khayyal MT, El-Ghazaly MA, Abdallah DM, Nassar NN, Okpanyi SN and Kreuter MH, 2002. Blood pressure lowering effect of an olive leaf extract (Olea europaea) in L-NAME induced hypertension in rats. *Arzneimittel-Forschung*, **52**, 797– 802.

Marrugat J, Covas MI, Fito M, Schroder H, Miro-Casas E, Gimeno E, Lopez-Sabater MC, de la Torre R and Farre M, 2004. Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation-a randomized controlled trial. *European Journal of Nutrition*, **43**, 140– 147.

Miro-Casas E, Covas MI, Farre M, Fito M, Ortuno J, Weinbrenner T, Roset P and de la Torre R, 2003. Hydroxytyrosol disposition in humans. *Clinical Chemistry*, **49**, 945– 952.

Moccetti T, Schmidlin CB, Aydogan C, Bradl B and Busjahn A, 2005. Effect of EFLA®943 in adult twins with mild hypertension. Internal Report.

Moschandreas J, Vissers MN, Wiseman S, van Putte KP and Kafatos A, 2002. Extra virgin olive oil phenols and markers of oxidation in Greek smokers: a randomized cross-over study. *European Journal of Clinical Nutrition*, **56**, 1024– 1029.

Ruano J, Lopez-Miranda J, Fuentes F, Moreno JA, Bellido C, Perez-Martinez P, Lozano A, Gomez P, Jimenez Y and Perez Jimenez F, 2005. Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. *Journal of the American College of Cardiology*, **46**, 1864– 1868.

Singleton VL and Rossi JA, Jr., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**, 144– 158.

Visioli F, Caruso D, Plasmati E, Patelli R, Mulinacci N, Romani A, Galli G and Galli C, 2001. Hydroxytyrosol, as a component of olive mill waste water, is dose- dependently absorbed and increases the antioxidant capacity of rat plasma. *Free Radical Research*, **34**, 301– 305.

Vissers MN, Zock PL, Roodenburg AJ, Leenen R and Katan MB, 2002. Olive oil phenols are absorbed in humans. *Journal of Nutrition*, **132**, 409– 417.

Weinbrenner T, Fito M, de la Torre R, Saez GT, Rijken P, Tormos C, Coolen S, Albaladejo MF, Abanades S, Schroder H, Marrugat J and Covas MI, 2004. Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. *Journal of Nutrition*, 134, 2314– 2321.

THE ONLY RELEVANT REFERENCE FOR (E)VOO ANALYSIS IS

[DE LA TORRE-CARBOT et al., 2005] and it says:

- Many studies have provided good information, and some of them have helped to clarify the structures of some phenolic compounds in oil.
- Nevertheless, because of the complexity of the wide group of secoiridoids, many of these phenolic compunds in OO remain unidentified.
- It is difficult to compare data within the literature, because of the lack of consistency: information is not only incomplete but sometimes contradictory as well.

THE ONLY RELEVANT REFERENCE FOR (E)VOO ANALYSIS

IT says as well:

- Nine basic models of ligstroside and oleuropein aglycons were found in the bibliography. Each model shares the same elenolic acid derivative ring structure.
- With final forms TyrOH & Tyr included
- Therefore: OLE & LIG DERIVATIVES are/were known at the time of SO! (So why … &)

SOME SCIENTIFIC REACTIONS TO THE HEALTH CLAIM

- The term 'olive polyphenols' is not the most accurate since only high-quality virgin olive oils (VOO) contain significant amounts of oleuropein and ligstroside aglycones and their conversion products.
- Also, the term 'olive oil' is generic and does not correspond to any dietary category.
- The inappropriate term 'polyphenols', which does not correlate with any of the secoiridoids in VOO and which are the main reason for the health claim.

[MASTRALEXI et al., 2014; TSIMIDOU et al., 2018; TSIMIDOU et al., 2019]

MINUTES OF THE GREX OO CHEM (link AnW-PolW)

Meeting was held at EC, AGRI, G4, subgroup olive oil chemists on 12 and 13 September 2017

- Point 11.1: Information and exchange of views on the health claim related to olive oil EU Regulation No 432/2012
- with the participation of Ms. Sabine Pelsser and M. Athanasos Raikos (DG SANTE)

MINUTES OF THE GREX OO CHEM

Lanfranco CONTE‘s intervention:

- The **inconsistency** of the claim itself for olive oil
- The uncertainty as regards the **phenolic compounds** to be determined
- The uncertainty as regards the **method** to be used for **determining** the **phenolic** compounds
- By then (2017), the IOC method was the **only official one** and was for the determination of the **total content** of polyphenols

Mrs. Pellser‘s reply:

- It is a Member states‘ issue – therefore, **MS must initiate** the **harmonization** process
- Future work – Oleum Project & IOC will take care of it.
  - (And they did, but …)

THE ACTIVITIES AFTER

WERE **analytical** and not political

- They tried to interpret and „reinvent“ the initially erroneous HC wording
- They were based on two premises:
  - If we cannot separate all the BP forms

  &
  - If we cannot calibrate all BP forms (even if separated)

THEN, it was thought

- Then we can hydrolyze the oil‘s BP, have only Tyr and TyrOH as a analyte, and quantify them, and the problem is solved (or almost …)

THE HYDROLYTIC ACTIVITIES

- HydL of the oil → HPLC of TyrOH & Tyr (HCl)
- HydL of the BP extract → HPLC of TyrOH & Tyr (H_2SO_4)
- HydL of the BP extract → GC of silanized TyrOH & Tyr (AcCl)

- HydL of the BP extract → UHPLC of TyrOH & Tyr– with Δ (H_2SO_4)
  (OLEUM Project approach)

OUR Opinion: consolidate them!

NoEX vs. EX: EX √

HCl HydL vs. H_2SO_4 HydL: HCl HydL √

HPLC vs. (U)HPLC: HPLC √

Δ Σ(TyrOH+Tyr) vs. Σ(TyrOH+Tyr): √ Δ Σ(TyrOH+Tyr)

THE ANALYTICAL CONSEQUENCES

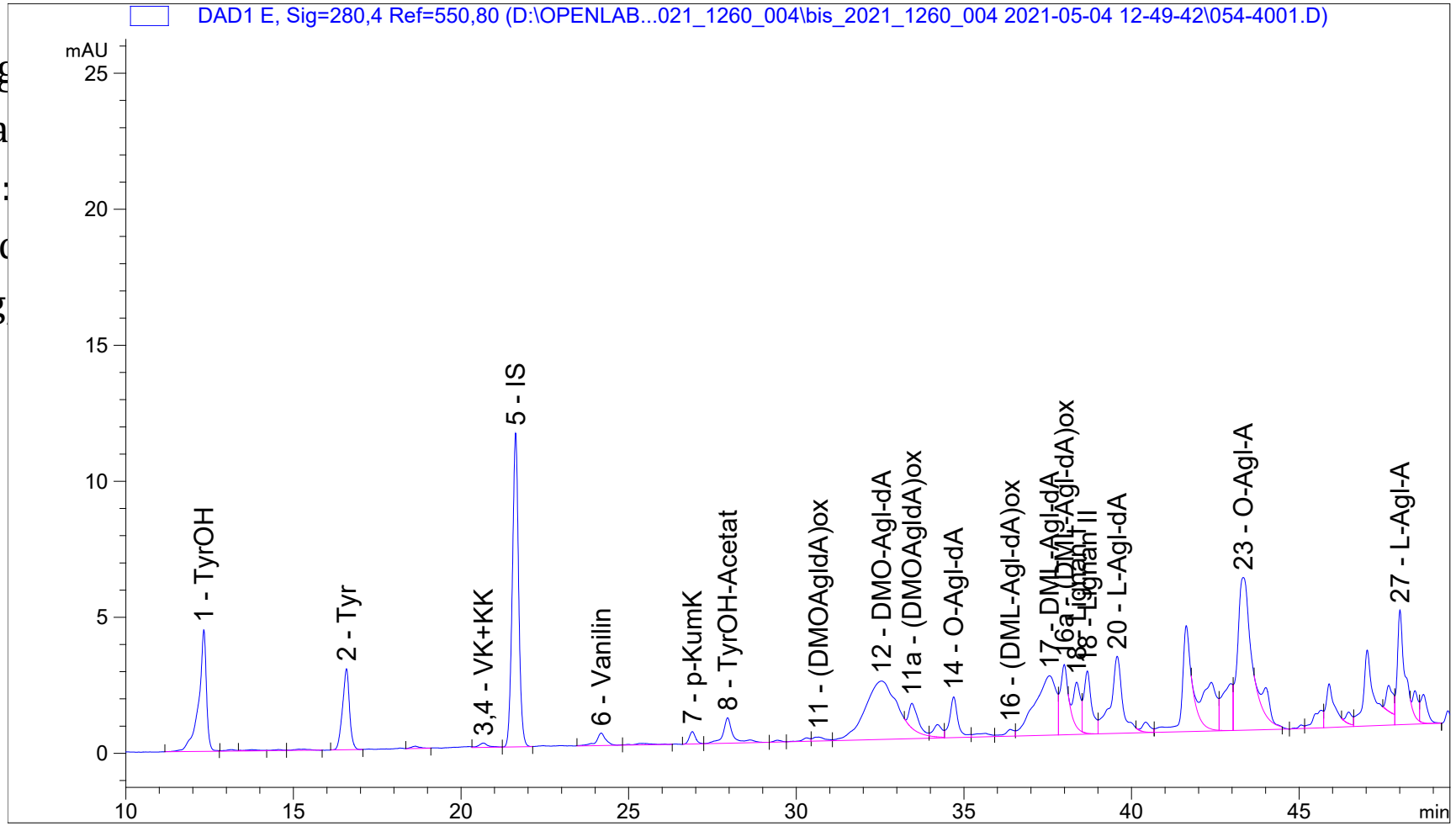
The research continued in trying to correlate the REAL BP content according to 432/2012 (which is ???) with the results of non-hydrolytic (U)HPLC, GC, and qNMR techniques:

- The IOC solution: trying to solve the problem with an alternative official method [TSIMIDOU, 2022; IOC, 2022]
- The Purcaro-Conte-Mariani solution: trying to find the correlation with the GC approach [PURCARO et al., 2014]
- The qNMR approach and reinterpretation of the IOC method [STAREC et al., 2021]

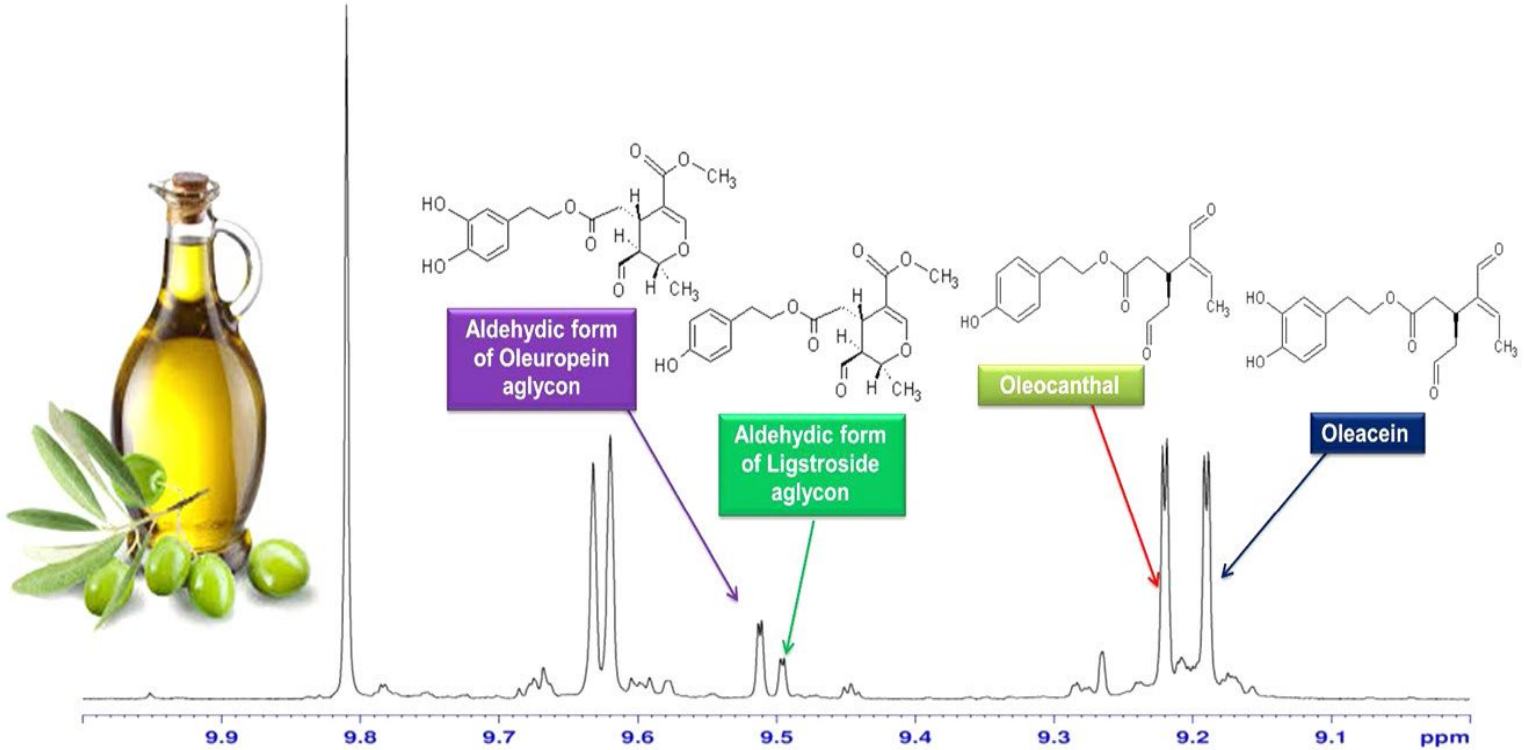
We need to rethink the original official IOC method

HPLC of BP by IOC method

- Kromatog
  - Ekstra
  - Ločba:
  - Detekc
  - 30 mg



[KARKOULA et al., 2014]



DOI: (10.1021/jf404421p)

FINAL REMARK – HOW TO SOLVE THE GAP – with a little help of MSR

- Instead of reinventing the possible analytical approach, let‘s correct the BP wording and include the exact BP terminology in the EU regulation.

- And then … solidify the analitics

THE INITIAL QUESTION WAS

Olive Oil Polyphenols Contribute to the Protection of Blood Lipids from Oxidative Stress" AND THE FATE OF EC REG. 432/2012: ARE THE BASIC TERMS AND THE FIGURES ANY CLEARER NOW?

THE INITIAL QUESTION WAS

Olive Oil Polyphenols Contribute to the Protection of Blood Lipids from Oxidative Stress" AND THE FATE OF EC REG. 432/2012: ARE THE BASIC TERMS AND THE FIGURES ANY CLEARER NOW?

THE ANSWER NOW IS

THE INITIAL QUESTION WAS

Olive Oil Polyphenols Contribute to the Protection of Blood Lipids from Oxidative Stress" AND THE FATE OF EC REG. 432/2012: ARE THE BASIC TERMS AND THE FIGURES ANY CLEARER NOW?

THE ANSWER NOW IS

- The basic terms ARE STILL CLEAR ONLY TO THE FOOD CHEMISTS

THE INITIAL QUESTION WAS

Olive Oil Polyphenols Contribute to the Protection of Blood Lipids from Oxidative Stress" AND THE FATE OF EC REG. 432/2012: ARE THE BASIC TERMS AND THE FIGURES ANY CLEARER NOW?

THE ANSWER NOW IS

- The basic terms ARE STILL CLEAR ONLY TO THE FOOD CHEMISTS
- The figures, unfortunately, follow the verse from the LC song:

THE INITIAL QUESTION WAS

Olive Oil Polyphenols Contribute to the Protection of Blood Lipids from Oxidative Stress" AND THE FATE OF EC REG. 432/2012: ARE THE BASIC TERMS AND THE FIGURES ANY CLEARER NOW?

THE ANSWER NOW IS

- The basic terms ARE STILL CLEAR ONLY TO THE FOOD CHEMISTS
- The figures, unfortunately, follow the verse from the LC song:

Whatever happened to my eyes
happened to your beauty!
Whatever happened to your beauty happened to me.

ACKNOWLEDGEMENTS

The authors and their LAB express deep gratitude to

Angelo Hlaj
Vanja Dujc
Sandi Babič
& numerous olive producers from the Istrian territory

who have helped promote the Istrian PDO Extra virgin Olive Oil.

REFERENCES 1

- ALAGNA, F.; MARIOTTI, R.; PANARA, F.; CAPORALI, S. et al. Olive phenolic compounds: metabolic and transcriptional profiling during fruit development. **Bmc Plant Biology**, 12, Sep 2012.
- CONTE, L., in **OLEUM Qualitá, tecnologia e sostenibilitá degli oli da olive**, Conte, L, Servili, M. (Eds.), Bologna, Edagricole, 2022.
- DE LA TORRE-CARBOT K, CHAVEZ-SERVIN JL, JAUREGUI O, CASTELLOTE AI, LAMUELA-RAVENTOS RM, NURMI T, POULSEN HE, GADDI AV, KAIKKONEN J, ZUNFT HF, KIESEWETTER H, FITO M, COVAS MI AND LOPEZ-SABATER MC, 2010. Elevated circulating LDL phenol levels in men who consumed virgin rather than refined olive oil are associated with less oxidation of plasma LDL. **Journal of Nutrition**, 140, 501– 508.
- DE LA TORRE-CARBOT, K.; JAUREGUI, O.; GIMENO, E.; CASTELLOTE, A. I. et al. Characterization and quantification of phenolic compounds in olive oils by solid-phase extraction, HPLC-DAD, and HPLC-MS/MS. **Journal of Agricultural and Food Chemistry**, 53, n. 11, p. 4331-4340, Jun 2005.

REFERENCES 2

- EUROPEAN COMMISSION. Regulation (EU) No 432/2012 of 16 May 2012 Establishing a List of Permitted Health Claims Made on Foods, Other than Those Referring to the Reduction of Disease Risk and to Children's Development and Health (2012) **Official Journal of the European Union**, L136, 1-40.
- IOC. Determination of phenolic compounds. Method COI/T.20/Doc. No 29/Rev.2/ 2022. Madrid, **International Olive Council**: 23. p.
- KARKOULA, E.; SKANTZARI, A.; MELIOU, E.; MAGIATIS, P. Quantitative Measurement of Major Secoiridoid Derivatives in Olive Oil Using qNMR. Proof of the Artificial Formation of Aldehydic Oleuropein and Ligstroside Aglycon Isomers. **Journal of Agricultural and Food Chemistry**, 62, n. 3, p. 600-607, Jan 2014.
- MASTRALEXI, A.; NENADIS, N.; TSIMIDOU, M. Z. Addressing Analytical Requirements To Support Health Claims on "Olive Oil Polyphenols" (EC Regulation 432/2012). **Journal of Agricultural and Food Chemistry**, 62, n. 12, p. 2459-2461, Mar 2014.

REFERENCES 3

- PANIZZI, L.; SCARPATI, M. L.; ORIENTE, G. Costituzione della oleuropeina, glucoside amaro e ad azione ipotensiva dell'ulivo, Nota II. **Gazz. Chim. Ital.** 90: 1449-1485 p. 1960.
- PODGORNIK, Maja (avtor, urednik, fotograf), FANTINIČ, Jakob (avtor, fotograf), BUČAR-MIKLAVČIČ, Milena (avtor, fotograf), VALENČIČ, Vasilij, BUTINAR, Bojan (avtor, fotograf), VODNIK, Dominik, GRAMC, Helena, KASTELEC, Damijana, FERLAN, Mitja, PINTAR, Marina. **Oljka, sušne razmere, tla in deficitno namakanje**. Koper: Znanstveno-raziskovalno središče, Inštitut za oljkarstvo, Annales ZRS, 2022. 102 str., ilustr. ISBN 978-961-7058-75-8. [COBISS.SI-ID 100049667]
- PURCARO, G.; CODONY, R.; PIZZALE, L.; MARIANI, C. *et al.* Evaluation of total hydroxytyrosol and tyrosol in extra virgin olive oils. **European Journal of Lipid Science and Technology**, 116, n. 7, p. 805-811, 2014/07/01 2014. https://doi.org/10.1002/ejlt.201300420.

REFERENCES 4

- SCIENTIFIC OPINION on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), “anti-inflammatory properties” (ID 1882), “contributes to the upper respiratory tract health” (ID 3468), “can help to maintain a normal function of gastrointestinal tract” (3779), and “contributes to body defences against external agents” (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006; **EFSA Journal**. 2011, 9, 2–225.
- STAREC, M.; CALABRETTI, A.; BERTI, F.; FORZATO, C. Oleocanthal Quantification Using H-1 NMR Spectroscopy and Polyphenols HPLC Analysis of Olive Oil from the Bianchera/Belica Cultivar. **Molecules**, 26, n. 1, Jan 2021.

REFERENCES 5

- TSIMIDOU, M. Z.; NENADIS, N.; SERVILI, M.; GONZALES, D. L. G. *et al.* Why Tyrosol Derivatives Have to Be Quantified in the Calculation of "Olive Oil Polyphenols" Content to Support the Health Claim Provisioned in the EC Reg. 432/2012. **European Journal of Lipid Science and Technology**, 120, n. 6, Jun 2018.
- TSIMIDOU, M. Z.; SOTIROGLOU, M.; MASTRALEXI, A.; NENADIS, N. *et al.* In House Validated UHPLC Protocol for the Determination of the Total Hydroxytyrosol and Tyrosol Content in Virgin Olive Oil Fit for the Purpose of the Health Claim Introduced by the EC Regulation 432/2012 for "Olive Oil Polyphenols". **Molecules**, 24, n. 6, Mar 2019
- TSIMIDOU, M. Z. A Critical Appraisal of the Separation Protocols Proposed for the Implementation of the Health Claim on "Olive Oil Polyphenols" (EC Regulation 432/2012). **Separations**, 9, n. 11, Nov 2022.