PI Caceu contracted the Linus Pauling Institute to conduct the main comparative analysis of ten (10) samples of extra virgin olive oil from different producers—two from Oregon, two from California, two samples from two distinct batches produced by the same Arizona producer (one batch made from estate-grown olives, the other made from olives grown on tribal land), one sample from Maui, and three imported olive oils (two Greek and one Italian, all three being very high quality and reportedly high in phenolic content). [1]

Total Polyphenol Content

TPC is an important measure of the health-benefit potential—as well as of freshness and quality, all of which have been correlated to significant health benefits in animals and humans. [2]

For the determination of total polyphenol content, a modification of the method by Fanali *et al.* (2018) was used. Olive oil samples were extracted as described using multiple hexane cleanup steps followed by extraction using MeOH/H_2O (3:2, *v/v*), vortexed and centrifuged for 10 min at 2,000 RPM. Hexane was discarded and the aqueous phase was used to determine the total polyphenol content by the Folin-Ciocalteau method. Samples were measured using UV spectrophotometry at 755 nm. Results were expressed as mg gallic acid equivalents (GAE)kg^{-1} sample. [3]

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[1] Other preliminary testing on samples of Oregon olive oils were contracted to two other labs: Eurofins in New Orleans, Louisiana (the U.S. division of a French multinational) and Modern Olives in Woodland, California (the U.S. division of an Australian multinational). These tests focused primarily on quality and freshness parameters, as a first threshold to clear before the core comparative analysis undertaken by the Linus Pauling Institute at Oregon State University.

[2] See https://pubmed.ncbi.nlm.nih.gov/?term=olive+oil+health+benefits

[3] Fanali C, Della Pasta S, Velmercati A, Dugo L, Russo M, Petitti T, Mondello L, de Gara L. Extraction, analysis, and antioxidant activity evaluation of phenolic compounds in differnet Italian extra-virgin olive oils. Molecules. 2018 Dec 8;23(12):3249.

Five Targeted Phenolic Compounds

The most important phenolic compounds were quantified. Targeted LC-MS/MS was used to quantitate tyrosol, hydroxytyrosol, oleuropein, oleacein, and oleocanthal.

Olive oil samples were extracted according to IOC COI/T.20/Doc No 29 with modification according to Celano (2018) using acetonitrile instead of methanol to decrease derivative formation of the secoiridoids. Phenolic extracts were injected using a Waters Acquity UHPLC coupled to a Waters Xevo TQD (Milford, MA). Separation was performed with a Waters Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.7 μM particle size) using gradient mobile phase delivery of acetonitrile containing 0.1% formic acid (MPB) and water containing 0.1% formic acid (MPA), as detailed by Lozano-Castellón with the modification of mobile phase B to acetonitrile. Phenolic compounds were quantitated with authentic standards (Sigma Aldrich, St Louis MO) and syringic acid used as the internal standard as detailed by IOC COI/T.20/Doc. No 29. Oleacein and oleocanthal were monitored in positive mode ESI, all other compounds were monitored in negative mode. [4]

[4] Determination of biophenols in olive oils by HPLC; COI/T.20/Doc. No 29; International Olive Council (IOC): Madrid, 2017.

Celano R, Piccinelli AL, Pugliese A, Carabetta S, de Sanzo R, Rastrelli L, Russo M. Insights into the analysis of phenoli secoiridoids in extra virgin olive oil. J Agric. Food Chem. 2018, 66, 6053-6063.

Lozano-Castellón J, López-Yerena A, Olmo-Cunillera A, Jáuregui O, Pérez M, Lamuela-Raventós RM, Vallverdú-Queralt A. Total analysis of the major secoiridoids in extra virgin olive oil: validation of an UHPLC-ESI-MS/MS method. Antioxidants. 2021, 10, 540.

Tocopherols

For the analysis of α- and γ-tocopherol, a modification of the method by Podda *et al.* (1996) was used. Briefly, ~25 mg of olive oil was saponified with alcoholic KOH, extracted with hexane, dried under nitrogen, resuspended in methanol, then injected into an HPLC system. The HPLC system consists of a Waters 2695 separations module with a 50µl sample loop. Tocopherols are detected using a LC-4B amperometric electrochemical detector (Bioanalytical Systems Inc., West Lafayette, IN, U.S.A.) with a glassy carbon working electrode, and a silver chloride reference electrode. The column used is a Phenomenex Synergi Hydro-RP, 150 × 4.6 mm, 4 µm particle size with a Phenomenex SecurityGuard precolumn, 4 × 3.0 mm. An isocratic mobile phase delivery system is used, with a total run time of 10 minutes. The mobile phase is 99:1 (v:v) methanol:water containing 0.1% (w:v) lithium perchlorate. The electrochemical detector is used in the oxidizing mode, potential 500 mV, full recorder scale at 500 nA. Peak areas are integrated using Waters Empower software package, and tocopherols are quantitated using authentic standards. A sample with known values was used for quality control. [5]

[5] Podda M, Weber C, Traber MG, Packer L. Simultaneous determination of tissue tocopherols, tocotrienols, ubiquinols and ubiquinones. J Lipid Res. 1996;37:893-901.

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FINAL REPORT

Olive oil polyphenols - May 22, 2024

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Sample	gT mg/kg	aT mg/kg	Total Tocopherols	Tyrosol mg/kg	Hydroxytyrosol mg/kg	Oleuropein mg/kg	Oleacein mg/kg	Oleocanthal mg/kg	Total Phenolics mg/kg
348	6.9	95.1	102.0	4.7	5.0	0.0	82.4	55.7	950.4
399	6.9	51.0	58.0	22.0	19.2	0.0	46.4	46.7	583.1
429	6.6	222.4	229.0	2.5	0.8	0.0	11.9	15.1	186.9
526	4.0	97.5	101.5	4.9	2.3	0.0	33.4	46.9	406.5
583	4.4	185.7	190.1	6.2	5.7	0.0	46.4	40.8	363.5
610	10.5	183.4	193.9	7.0	2.1	0.0	51.3	82.2	463.0
688	6.3	102.7	109.0	7.6	4.4	0.0	53.4	74.7	498.4
696	4.0	94.5	98.5	1.5	0.6	0.0	27.0	23.7	213.3
851	4.7	116.0	120.7	4.9	1.3	0.0	34.3	64.1	358.7
913	6.1	86.0	92.1	1.8	1.7	0.0	147.4	79.6	576.9
Olive leaf powder	11.0	152.4	163.4	42.7	569.3	463.8	0.0	1.7	19763.4

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Results

In terms of TPC, one the two samples of olive oil produced from olives grown in Oregon (348) showed the highest levels—by a significant margin: 950.4 mg/kg versus 583.1 and 576.9 for the second- and third-ranked samples (399, from California, and 913, from Oregon, respectively). While more testing is required to document clearly confirmed trends over a number of years, one indication is that this particular olive orchard in Oregon has planted very specific olive cultivars (with a focus on high-quality and less quantity) and has implemented highly-sustainable cultural practices for a number of years; also, this grower practices hand-picking (labor intensive yet very good for the quality of the fruit) and harvests earlier in the season (again, sacrificing yield but gaining in terms of quality and total phenolic content).

In terms of specific phenolic compounds, hydroxytyrosol is worth emphasizing because it is the compound for which a limited health claim exists for olive oil—not in the U.S. but in the EU where the FDA's equivalent, the EFSA, has allowed a very limited 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. [6]

The sample from California (399) scored particularly high, followed by samples from Arizona (583) and Oregon (348).

In terms of tocopherols, a sample from California (610) and the two samples from Arizona (429 and 583) showed the highest levels, by a significant margin. The levels of tocopherols are interesting to emphasize because they have vitamin E activity.

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[6] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R0432&from=EN

Across most parameters, olive oils from the Western region of the U.S. scored higher—significantly higher—than imported oils that were marketed as high-phenolic.

The main lesson—and strongest recommendation to U.S. olive oil producers is to begin to emphasize the high-phenolic character of their oils and to link it to the quality of the oils and to the sustainable practices undertaken in the olive orchards.