Determination of Biophenols in Olive Oil

Li Sheng (lsheng@eplbas.com)

EPL BAS™

Abstract

Vegetable oils contain various antioxidants, including vitamin E and unsaturated fatty acids. Some of these oils, especially olive oil, contain another group of antioxidant – phenolic compounds. The health benefits from the phenolics have recently been published with the rising recommendation of communicating these effects on food labels. As an official testing method is unavailable from AOCS and AOAC, we developed an HPLC method based on the biophenol method of the International Olive Council. Many analytical standards are commercially unavailable for biophenols, and neither retention time nor capacity factor can be used in chromatograms for biophenol identification. Therefore we identified a set of available standards with different retention times to spread across the chromatogram in addition to an internal standard. The biophenol profile can thus be established referring to these standards. More than 20 biophenols were included in this method. Different brands of olive oil and several types of vegetable oils were sampled and tested. Extra virgin olive oil was found to have the highest content while fewer biophenols were found in processed olive oil and other vegetable oils. As for the other vegetable oils different biophenol profiles were also observed. This method has great potential in olive oil authenticity testing, and in quality control to differentiate substandard olive oils.

Conclusions

An updated analytical method for the determination of biophenols in olive oils was developed and successfully used for various types of commercial olive oils.
The In++ternational Olive Council specified 31 biophenols and quantified the total. EPL method achieved the goal with precision and accuracy.

Reference

1. International Olive Council. (2009). Determination of Biophenols in Olive Oils by HPLC. COI/T.20/Doc No 29.

Introduction

Olive oil is often considered characteristic of the Mediterranean diet. It contains a high level of biophenols, which have been identified to have anti-oxidant, anti-inflammatory and anti-microbial activities. An accurate test of the biophenols is important. The method we developed was based on the International Olive Council method COI/T.20/Doc No 29 and was vigorously tested for efficiency and robustness for olive oil testing.

Materials and Methods
Standards (Sigma-Aldrich, St. Louis, MO):

1. 2- (4 - hydroxyphenyl) ethanol (tyrosol)
2. 3,5 dimethoxy 4- hydroxy benzoic acid (syringic acid)
3. Ferulic acid
4. Oleuropein
5. Cinnamic acid
6. Luteolin
7. Apigenin
8. Other Standards (optional):
   a. Vanillic Acid
   b. Caffeic Acid
   c. Vanillin
   d. Coumaric Acid

Test Matrices - Commercially available oils

Equipment

1. High-performance ternary gradient liquid chromatograph (HPLC), equipped with C18 reverse-phase column (4.6 mm x 25 cm), type Spherisorb ODS-2 5 µm, 100 Å, with spectrophotometric UV detector at 280 nm and integrator.
2. Others as listed in method COI/T.20/Doc No 29.

Sample Preparation - Samples were prepared according to COI/T.20/Doc No 29.

Results and Discussion - HPLC Chromatogram of Olive Oil Samples

The reference chromatogram (Fig. 1) listed about 30 biophenols (Table. 1)

- Internal standard – syringic acid (peak 5)
- External standard - tyrosol (peak 2) and syringic acid (peak 5)
- 4 biophenols eluted before external standards
- 25 biophenols eluted after external standards
- 20 biophenols identified within un-resolved peaks

Figure 1
Reference chromatogram found in method COI/T.20/Doc No 29 (biophenols profile present in an extra virgin olive oil).

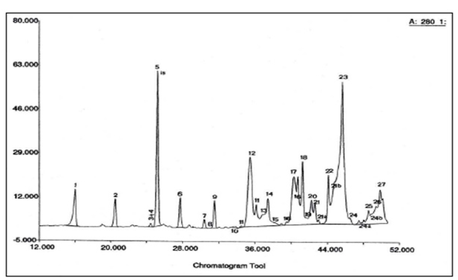


Table 1.

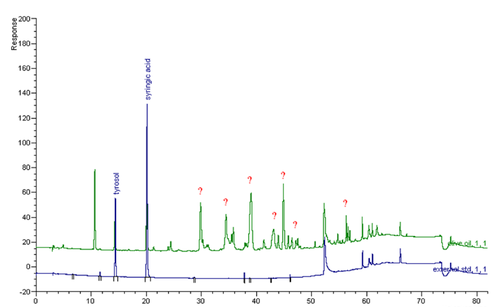
Identification of biphenols peaks. Maximum absorbance (max UV abs) values and relative retention times (RRT)*1

Peak No	Biophenols	RRT*	Max. UV abs. nm
1	Hydroxytyrosol	0.62	230-280
2	Tyrosol	0.8	230-275
3	Vanillic acid	0.96	260
4	Caffeic acid	0.99	325
5	Syringic acid (internal standard)	1	280
6	Vanillin	1.1	310
7	Para-coumaric acid	1.12	310
8	Hydroxytyrosyl acetate	1.2	232-285
9	Ferulic acid	1.26	325
10	Ortho-coumaric acid	1.31	325
11;11a	Decarboxymethyl oleuropein aglycone, oxidised dialdehyde form	-	235-280
12	Decarboxymethyl oleuropein aglycone, dialdehyde form	1.45	235-280
13	Oleuropein	1.48	230-280
14	Oleuropein aglycone, dialdehyde form	1.52	235-280
15	Tyrosyl acetate	1.54	230-280
16;16a	Decarboxymethyl ligstroside aglycone, oxidised dialdehyde form	1.63	235-275
17	Decarboxymethyl ligstroside aglycone, dialdehyde form	1.65	235-275
18	Pinoresinol, 1 acetoxy-pinoresinol	1.69	232-280
19	Cinnamic acid	1.73	270
20	Ligstroside aglycone, dialdehyde form	1.74	235-275
21;21a;21b	Oleuropein aglycone, oxidised aldehyde and hydroxylic form	-	235-280
22	Luteolin	1.79	255-350
23	Oleuropein aglycone, aldehyde and hydroxylic form	1.87	235-280
24;24a;24b	Ligstroside aglycone, oxidised aldehyde and hydroxylic form	-	235-275
25	Apigenin	1.98	230-270-340
26	Methyl-luteolin	-	255-350
27	Ligstroside aglycone, aldehyde and hydroxylic form	2.03	235-275

Olive oil sample analysis (Fig. 2)

- All biophenols: retention times and relative retention times (RRT) change when different HPLC systems, HPLC columns, and HPLC temperatures were used
- 20 biophenols eluted after the standard peaks – difficult to identify
- Quantification of total biophenols difficult – how many component peaks to be included?

Figure 2. HPLC Chromatograms of an olive oil sample. Top: olive oil; bottom: external standard.



Adding Standards to Identify Biophenols

Additional biophenol standards selected based on the reference chromatogram

- Adding biophenol standard – 5 critical, 4 optional (Fig. 3)
- Select standards spread across the chromatogram (Fig. 3)
- All standards peaks were base-line resolved (Fig. 4)
- Biophenol peak identification easily achieved using the set of biophenol standards (Fig. 5)
- All chosen standards are commercially available

Figure 3. Choosing required standards.

I Std = internal standard;
E Std = external standard;
O = required standards;
□ = optional standards.

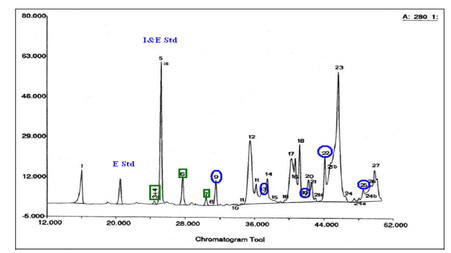


Figure 4. HPLC chromatogram of the select standards.
Top: mixed standard; bottom: external standard.

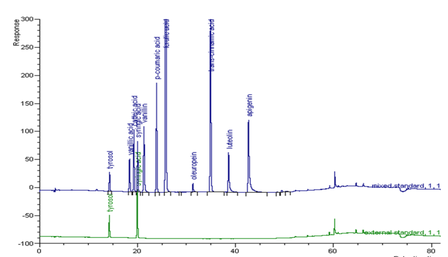
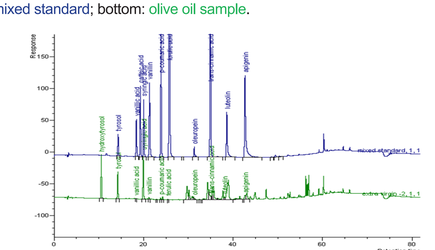


Figure 5. HPLC chromatograms of the mixed standard and an extra virgin olive oil sample.

Top: mixed standard; bottom: olive oil sample.



Biophenol Test Result of Olive Oils

Added biophenol standards facilitate peak identification and quantification

- Different brands of extra virgin olive oils have similar yet distinctive biophenol profiles (Fig. 6)
- Correct identification is critical for quantification (Table 2)
- Excellent precision and accuracy demonstrated in biophenol quantification work (Table 2)

Figure 6. HPLC chromatogram of extra virgin olive oils.
Top: US extra virgin olive oil; middle: imported extra virgin olive oil brand 1; bottom: imported extra virgin olive oil brand 2.

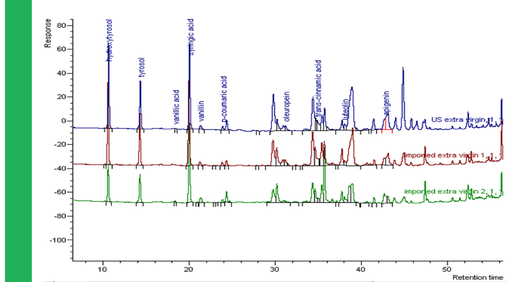


Table 2. Analytical Result of Biophenol Content (mg/Kg) in Extra Virgin Olive Oils

Sample	Biophenol Content (mg/Kg)	Average Content (mg/Kg)	STDEV	CV (%)
Imported Extra Virgin 1-1	295.80	293.44	5.58	1.90
Imported Extra Virgin 1-2	297.45			
Imported Extra Virgin 1-3	287.07			
US Extra Virgin -1	323.80	327.00	6.77	2.07
US Extra Virgin -2	334.78			
US Extra Virgin -3	322.42			

Biophenol Profile of Oils
Biophenol analysis of various oils (Fig. 7)
- Biophenol profiles of various types of oils are different
- Biophenol content is negligible in vegetable oil and canola oil
- Biophenol content is much lower in pure olive oil than extra virgin olive oil

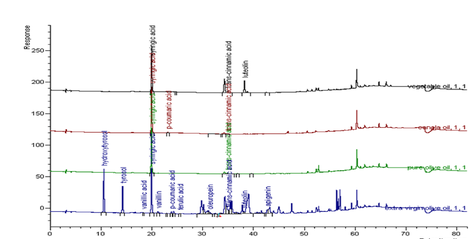



Figure 7. HPLC Chromatograms of Oils.
From the bottom: extra virgin olive oil, pure olive oil, canola oil, vegetable oil.