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Report

Quantification of Tyrosol and Hydroxytyrosol in Oliveoil

Customer:	Dr. Petra Pforr
Sample description:	**Olive oil Villa Santo Stefano, Olio Extravergine di oliva biologico, LUCCA**
Processing duration:	20.12.2023
Coworkers:	Marlene Weber, M.Sc

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1. Summary

The aim of this analysis is to determine the tyrosol and hydroxytyrosol content of olive oil produced by *Villa Santo Stefano, Olio Extravergine di oliva biologico, LUCCA*. The samples were analysed after extraction with 60% methanol (direct analysis) and after hydrolysis with hydrochloric acid. Neither hydroxytyrosol nor tyrosol could be quantified in the directly analysed samples (LOQ = 0.5 ppm). The following table shows the results of the quantitative analysis of hydroxytyrosol and tyrosol in mg per 20 g including relative standard deviations, as well as the total amount.

Table 1: *Content of Tyrosol and Hydroxytyrosol in mg/20 g of olive oil sample LUCCA after Hydrolysis and after Extraction LOQ = 0,5 mg/l (measurement concentration).*

	Hydroxytyrosol mg/20 g	Tyrosol mg/20 g	Total Amount mg/20 g
Hydrolysis	1,85 ± 2,7%	3,36 ±2,6%	5,21
Extraction	n.q.	n.q.	-

n.q. = not quantifiable

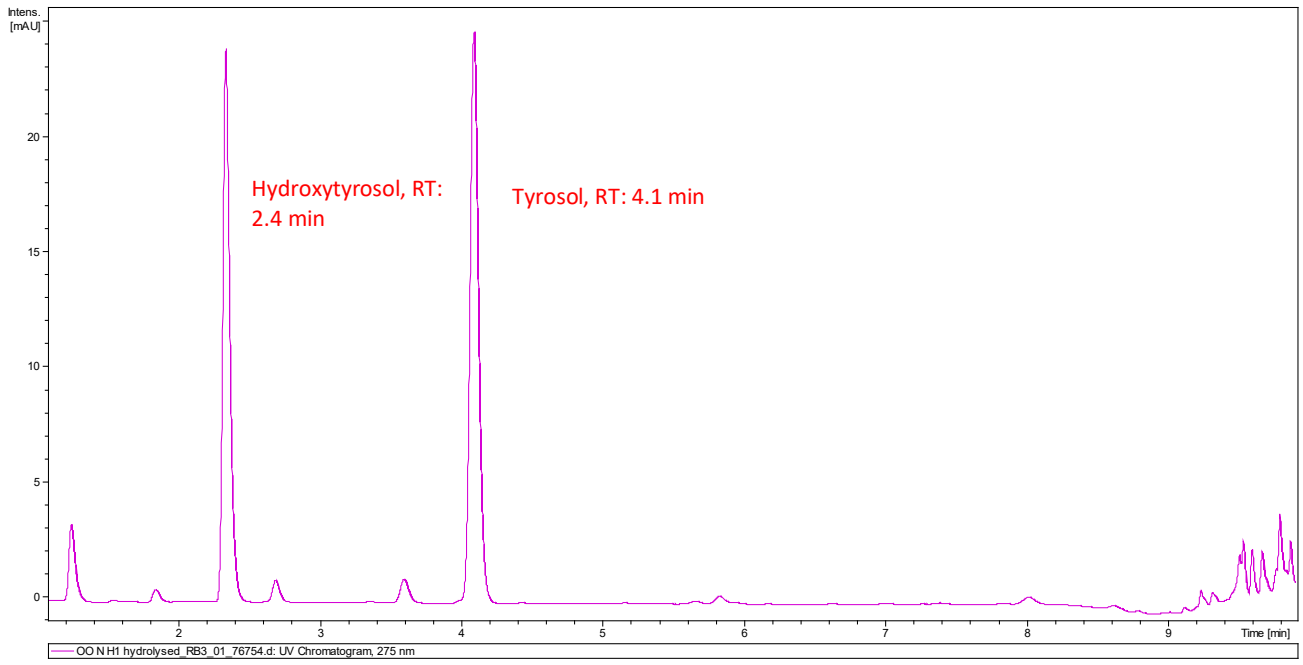

Figure 1: UV-Chromatogram at 275 nm of sample with hydrolysis; Agilent RRHD Zorbax C18 (2.1 x 100 mm, 1.8 µm) column, mobile phase acetonitrile (B) and 0.1 % formic acid (FA) in water (A).

2. Methods and procedure

For each sample, two replicates were analysed, both for the direct determination and the hydrolysis, followed by two analytical measurements in each case. The analytes were quantified via UV (275 nm) and the signals were identified via mass spectrometry. The instrument parameters were chosen according to Mair *et al* Separations 2023 10 268 (DOI 10.3390/separations10040268). The quantifications were performed using recently supplied analytical standards.

2.1. Calibration Line

A stock solution with a concentration of 100 ppm tyrosol and 100 ppm 3-hydroxytyrosol was prepared. For this purpose, tyrosol (Sigma-Aldrich 79058-500MG-F) and 3-hydroxytyrosol (Sigma-Aldrich 91404-5MG) were partially dissolved in 5% methanol (v/v). The stock solutions were diluted to final concentrations of 0.1, 0.5, 1, 5, and 10 ppm, resulting in a 5-point calibration curve. Each calibration standard was measured in the form of quadruple determinations. The coefficients of determination obtained were 0.9997 for 3-hydroxytyrosol and 0.9997 for tyrosol.

2.2. Sample Preparation

Acidic Hydrolysis

Approximately 50 mg of each olive oil (duplicates) was weighed in a 2 ml Eppendorf reaction tube. After the addition of 1 ml of 0.5 M hydrochloric acid, the mixture was shaken at 80 °C at 1,400 rpm for 90 min in a thermomixer (Eppendorf Thermomixer comfort). Afterwards it was centrifuged at 14,000 rpm and 5 °C. for 3 min (Eppendorf 5430R, FA-45-30-11 rotor) and an aliquot of the lower, aqueous phase was subjected to HPLC-UV-MS analysis.

Direct Quantification of Tyrosol and 3-Hydroxytyrosol

Approximately 50 mg of each olive oil (duplicates) was weighed into a 2 ml Eppendorf reaction tube. After adding 500 µL hexane and 350 µL 60% methanol as an extraction solvent, the mixture was shaken at 20 °C, 1,400 rpm for 5 min in a thermomixer (Eppendorf Thermomixer comfort). The mixture was then centrifuged at 14,000 rpm and 20 °C. for 2 min (Eppendorf 5430R, FA-45-30-11 rotor). The oil phase was subsequently transferred to another Eppendorf reaction tube and 350 µL 60% methanol (v/v) were added. The extraction step was repeated three times. In the final step, the aqueous phase was retained, and the three extracted samples were collected in an Eppendorf tube. As before, 500 µL hexane was added and shaken for 5 min and centrifuged for 2 min. The lower phase was retained, and the extracted sample was transferred into a 2 ml volumetric flask and brought to volume with deionized water. Subsequent to vortexing the samples, they were subjected to HPLC-UV-MS analysis.

2.3. HPLC-UV Parameters

The quantifications were performed on a Thermo Scientific Ultimate 3000 UHPLC system equipped with an LPG-3400SD pump, a WPS-3000TSL autosampler, a TCC-3000SD column oven and a VWS-3100 UV detector (wavelength 275 nm). An Agilent RRHD Zorbax C18 column (2.1 x 100 mm, 1.8 µm) was used for the separation of the analytes, the flow rate was 0.4 ml/min, the injection volume was 5 µl, and the separation was carried out at a column temperature of 50°C. 0.1% formic acid in H_2O was used as eluent A and acetonitrile as eluent B. The following separation gradient was employed: 3% B to 10% B in 7 min, to 100% B within 1 min, 2 min 100% B, 100% B to 3% B in 1 min, 4 min 3% B.

3. Results

3.1. Quantification of Tyrosol and Hydroxytyrosol

Table 1 illustrates the analysed olive oil with and without hydrolysis, the results show the content of hydroxytyrosol and tyrosol in mg per 20 g, as well as the total amount of both compounds. The tyrosol and hydroxytyrosol content for the extracted samples was found to be below the limit of quanitifcation in mg/l (measurement concentration).

Table 2: *Content of Tyrosol and Hydroxytyrosol in mg/20 g of olive oil sample LUCCA after Hydrolysis and after Extraction LOQ = 0,5 mg/l (measurement concentration).*

	Hydroxytyrosol mg/20 g	Tyrosol mg/20 g	Total Amount mg/20 g
Hydrolysis	1,85 ± 2,7%	3,36 ± 2,6%	5,21
Extraction	n.q.	n.q.	-

n.q. = not quantifiable

The following figure shows the chromatogram of sample without hydrolysis. As the results of table 1 have already shown, the signal for tyrosol and hydroxytyrosol was found to be below the limit of quantification. Therefore, only one chromatogram is shown for illustrative purposes.

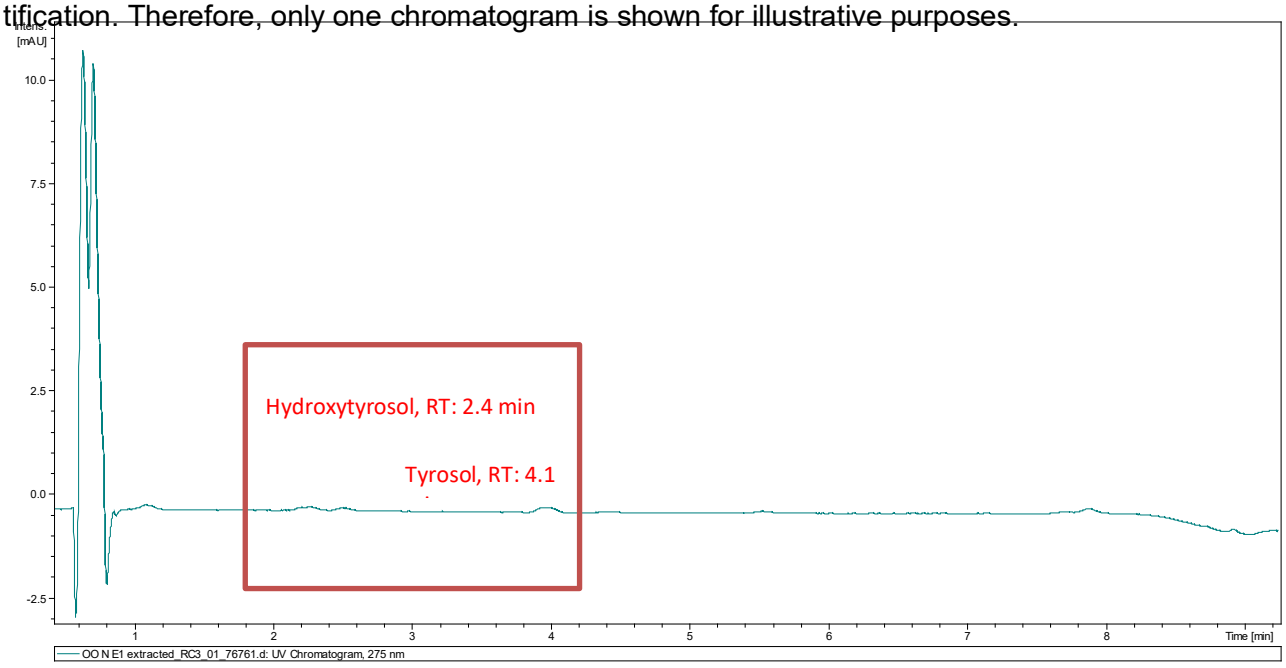

Figure 2: *UV-Chromatogram at 275 nm of sample without hydrolysis; Agilent RRHD Zorbax C18 (2.1 x 100 mm, 1.8 µm) column, mobile phase acetonitrile (B) and 0.1 % formic acid (FA) in water (A)*

The following figure shows the UV-chromatograms of the hydrolyzed olive oil samples. The retention times of hydroxytyrosol and tyrosol are 2.4 min and 4.1 min, respectively.

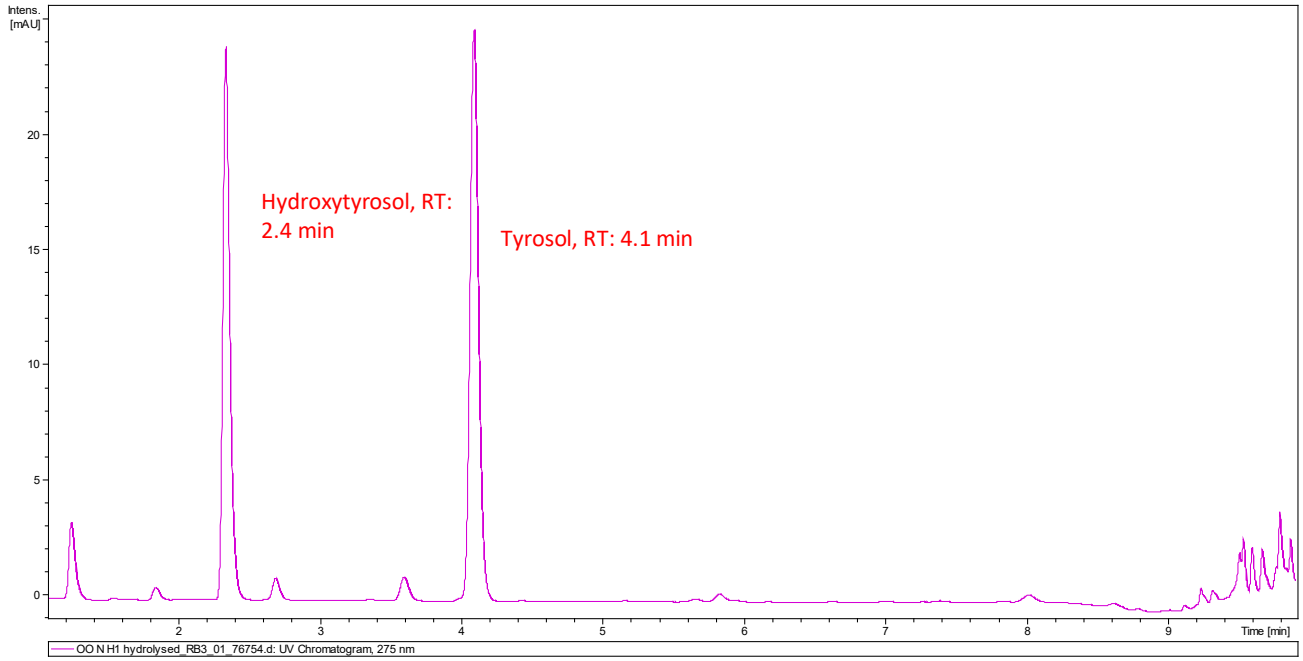

Figure 3: UV-Chromatogram at 275 nm of sample with hydrolysis; Agilent RRHD Zorbax C18 (2.1 x 100 mm, 1.8 µm) column, mobile phase acetonitrile (B) and 0.1 % formic acid (FA) in water (A).

3.1 Standard mix – calibration - tyrosol and hydroxytyrosol

Figure 3 shows an overlay of the UV chromatograms of the standard mix of tyrosol and hydroxytyrosol at a UV wavelength of 275 nm. The calibration solution was diluted to final concentrations of 0.1, 0.5, 1, 5, and 10 ppm, resulting in a 5-point calibration curve.

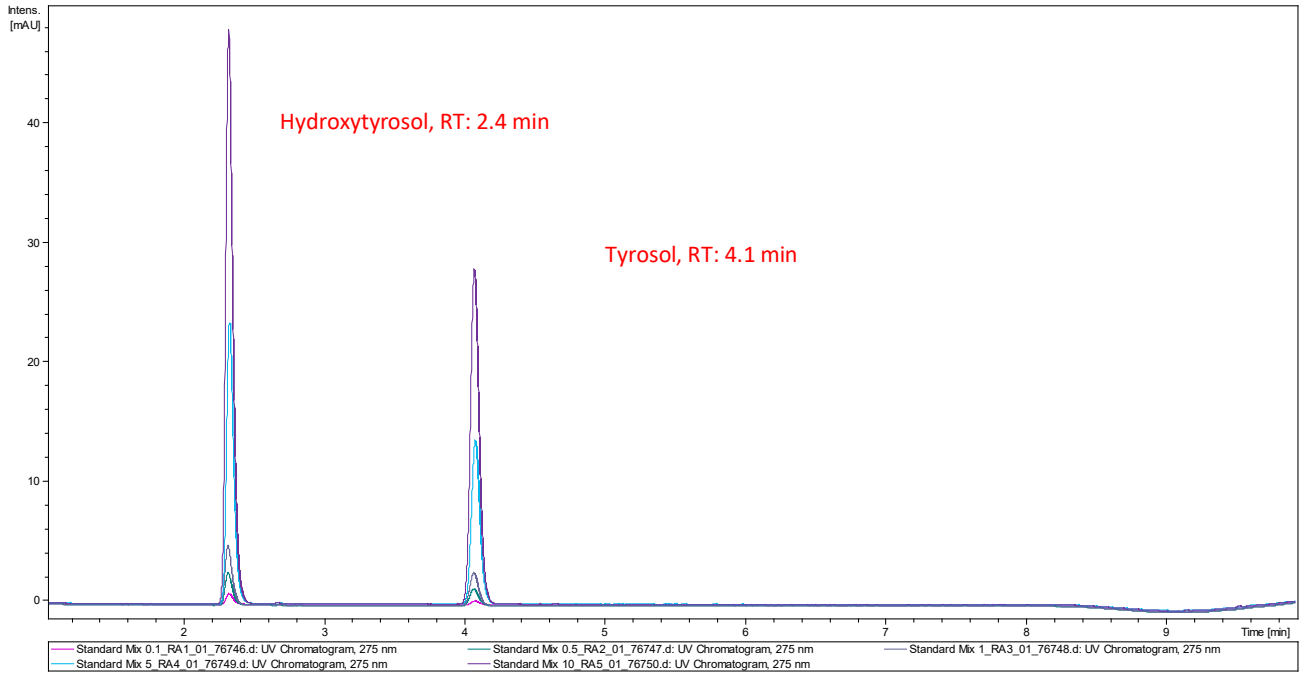

Figure 3: *UV-Chromatogram at 275 nm standard mix tyrosol and hydroxytyrosol 0.1-10 ppm; Agilent RRHD Zorbax C18 (2.1 x 100 mm, 1.8 µm) column, mobile phase acetonitrile (B) and 0.1 % formic acid (FA) in water (A).*

4. Conclusion

The quantitative analysis of the olive oil sample (Olio Extravergine di oliva biologico, LUCCA) resulted in a concentration of 5.21 mg EFSA-health-claim related polyphenols per 20g olive oil, thus the oil bears the claim "Olive Oil Polyphenols" regarding EC Regulation 432/2012.