Size exclusion chromatography in olive oil quality control

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Olive oil is a natural fruit product of fine aroma, pleasant taste, and high nutritional value.[1] It is composed primarily of triglycerides and minor constituents such as free fatty acids, hydrocarbons, antioxidants, and flavor compounds. These minor constituents are important for the stability and flavor of the oil. Their quantitative analysis is a major determinant of the authentication of various olive oil types.

Numerous adulterants[2] have been found in olive oil. Adulteration takes place not only through accidental contamination during the stages of oil processing, but more often by deliberate mislabeling of less expensive products or admixtures containing less costly oils for the purpose of financial gain. Monitoring the genuineness of olive oil is therefore very important from both commercial and health aspects. Quality and authenticity criteria for various olive oil types are described in detail in the Norm of the Codex Alimentarious[3] and the EC Commission Regulation 2568/91.[4] Several physical and chemical tests have been used to detect adulteration of olive oil by low-grade oils.[1,5,6] Recently, these tests have been complemented or substituted by many modern instrumental techniques such as HPLC, chiral chromatography, stable carbon isotope ratio analysis (SCIRA), nuclear magnetic resonance (NMR) spectrometry, and Fourier transform-midinfrared (FT-MIR) spectroscopy.[2,7]

Using gel permeation chromatography (GPC), olive oil minor constituents (diglycerides, free fatty acids, oxidation products, and dimers) can be fractionated and therefore quantified. Olive oil may have to be stored for many months. If specific precautions are not taken against deterioration, storage will cause an increase in acidity due to the action of lipase and the development of rancidity due to oxidation involving changes in aroma and taste of the oil. GPC analysis can be a helpful tool in determining changes due to storage and in evaluating the effectiveness of various materials used for packaging vegetable oils. In addition, GPC can be used in a complementary manner or independently for detecting olive oil adulteration.

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Using GPC, vegetable oils were analyzed after deep fat frying or following exposure to elevated temperatures (>150 °C).[9,10] The aim of the present work was to develop a rapid and effective method with which to evaluate the quality of fresh olive oil and olive oil stored under conditions closer to those prevailing in supermarkets and in the homes of consumers. The method was further validated for olive oil samples stored under diffused light for six months and in the dark for 18 months.

Materials and samples

Samples

Virgin olive oil samples purchased from the supermarket were used. Analysis of initial quality characteristics (acidity values, peroxide values, and extinction coefficients K_{232} and K_{270}) confirmed that all samples belonged to the category of the best-quality olive oil designated "extra."[4]

Reagents and standards

Tetrahydrofuran (THF) was purchased from **Merck KGaA** (Darmstadt, Germany) and was distilled over potassium prior to use. Polystyrene ReadyCal standards of varying molecular weights were provided by **PSS Ltd.** (Mainz, Germany).

Apparatus

The HP 1100 isocratic HPLC system (**Agilent Ltd.,** Waldbronn, Germany) consisting of an HP UV detector and Shodex RI-71 refractive index (RI) detector (**Showa Denko Ltd.,** Düsseldorf, Germany) was used. An SDV 100-Å (300 × 8 mm i.d.) column with 5-µm packing (**PSS Ltd.**) was used. The flow rate was 1 mL/min and the sample injection volume was 20 µL. Data were collected and processed using PSS-WinGPC 6.20 software (**PSS Inc.,** Silver Spring, MD).

Preparation of sample and calibration curve

Suitable amounts of oil samples were dissolved in THF to give a final concentration of 5 mg/mL, and 20 µL of the solution was injected into the GPC system. The calibration curve of the standards was obtained by dissolving a suitable amount of polystyrene mixture in 1 mL THF to obtain a concentration of 0.15%. The solution was then injected into the GPC system. Three different standard polystyrene mixtures of varying molecular weights were used. These mixtures are characterized by a narrow molecular-weight distribution and are commercially available as Polystyrene ReadyCals.

Chromatographic separation and identification

The elution protocol was designed to achieve adequate separation of triglycerides, diglycerides, monoglycerides, free fatty acids, and oxidation products within a reasonable length of time. THF was used as the eluent at a flow rate of 1 mL/min. Compounds were detected using the UV and RI detectors. Identification was based on the spectral characteristics of the compounds, elution volumes, and standard calibration curve in the range 162–2,180,000 D.

Stability study

In order to monitor quality changes during storage, olive oil samples were stored under diffused light for six months and in darkness for 18 months. Sample selection for the storage study was based on the samples' initial quality characteristics. Transparent glass bottles of 100-mL capacity were filled with oil samples and were tightly sealed with no headspace. One set of samples was stored under diffused light at about 25 °C, while another set was stored in the dark at about 22 °C. At six-month intervals, quality of samples was determined by GPC.

Results and discussion

Chromatographic system

GPC separations separate molecules according to their size. To avoid adsorption during separation, a porous chromatographic stationary phase and an appropriate mobile phase solvent are needed. The system used in this study was equipped with two SDV 100-Å, 5-µm, 8 × 300 mm i.d. columns that provided 90,000 theoretical plates as determined with 2,6-di-tert-butyl-4-methylphenol (BHT) in THF. The chromatographic asymmetry factor was good. The molecular-weight calibration curve (molecular weight versus elution volume) of the system was obtained using polystyrene standards of narrow molecular-weight distribution (ReadyCals) and WinGPC 6.20 software. The pore diameter of the SDV 100-Å column was determined to be 5.4 nm using the PSS-PoreCheck software (**PSS Ltd.**).[11]

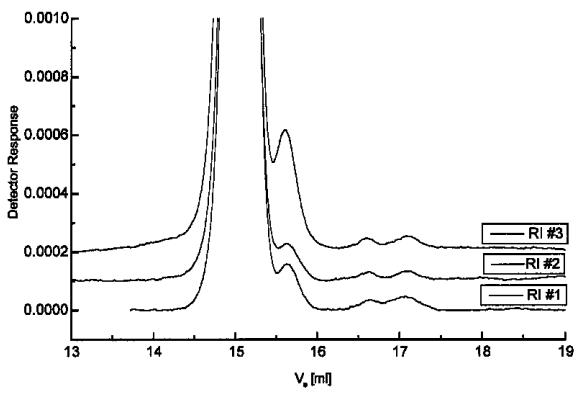

Figure 1 *GPC-RI analysis of the original olive oil samples (1, 2, 3). Eluent: THF, 1.00 mL/min. Injection volume: 20 µL. Sample concentration: 5 mg/mL eluent. Detector response: highest signal × 10.*

Analysis of original samples

Three different virgin olive oil samples (sample 1, 2, and 3) belonging to the category "extra" were studied. The molecular-weight fractions were separated and analyzed by GPC-RI detection and the results are presented in *Figure 1*. High molecular weight compounds are observed at low elution volumes. The main peak at 15.0 mL represents triglyceride, and the smaller peak at 15.6 mL is diglyceride. The weak peak at 16.6 mL is possibly monoglyceride, while the peak at 17.2 mL corresponds to hydrolyzed acid.

Stability under diffused light

The GPC-RI elution profile of the samples after six months' storage in diffused light (*Figure 2*) was very similar to that of the original samples (Figure 1) and did not provide substantial information on quality changes. This is due to the poor sensitivity of the RI

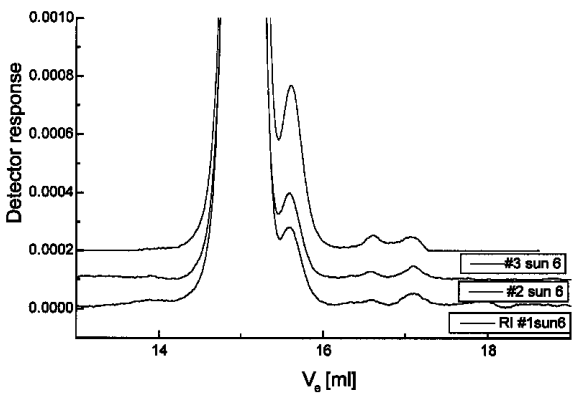

Figure 2 *GPC-RI analysis samples stored under diffused light for six months (1sun6, 2sun6, and 3sun6). Eluent: THF, 1.00 mL/min. Injection volume: 20 µL. Sample concentration: 5 mg/mL eluent. Detector response: highest signal ×10.*

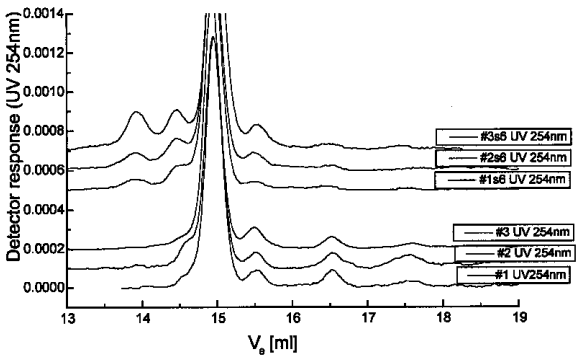

Figure 3 *GPC-UV (254 nm) comparison of original samples (lower elution profile) with samples stored under diffused light for six months (upper elution profile). Eluent: THF, 1.00 mL/min. Injection volume 20 µL. Sample concentration: 5 mg/mL eluent. Detector response: largest signal — 0.0014 rel. ads. units.*

detector. Suppressing the main signal (triglyceride) by means of chromatographic sample enrichment on a silica phase may enhance the use of RI for quantification[12] (investigation in progress). Alternatively, changes in the quality of aged samples were determined successfully using UV detection fixed at λ = 254 nm. The method is sensitive enough for the qualitative and semiquantitative analysis of product changes. However, it cannot be used for complete quantitative determination of products having different chromophoric groups. *Figure 3* depicts the GPC-UV profile of the samples (1s6, 2s6, and 3s6) stored under diffused light for six months. A remarkable difference in quality, in comparison to the original samples, is evident. High-molecular-weight compounds are present as a result of photooxidation. The retention volumes 13.9 and 14.5 mL correspond to the dimer and oxidation product, respectively.

Stability in darkness

To evaluate quality changes during storage in the dark, GPC profiles of samples stored for 6, 12, and 18 months were compared to those of the original samples. Again, it was noted that RI detection was not sensitive enough to detect quality changes due to storage. GPC-UV detection in the dark for 6 and 12 months was achieved (figures not shown), but the changes were less significant than those shown in Figure 3. A relatively high degree of quality deterioration was observed after 18 months (*Figure 4*, samples 1d18, 2d18, and 3d18). The oxidation product (14.5 mL) and the dimer product (14 mL) increased. However, free fatty acid (17.5 mL) and diglyceride (16.6 mL) remained relatively unchanged. It is interesting to note that the deterioration in quality was greater for samples stored under diffused light for six months than for samples stored in the dark for 18 months. This could be attributed to the fact that in the presence of light, vegetable oils undergo photooxidation, whereas in the dark, autooxidation occurs. Olive oil is resistant to autooxidation due to its high content of natural antioxidants. However, it is very sensitive to photooxidation, which is more detrimental than autooxidation.

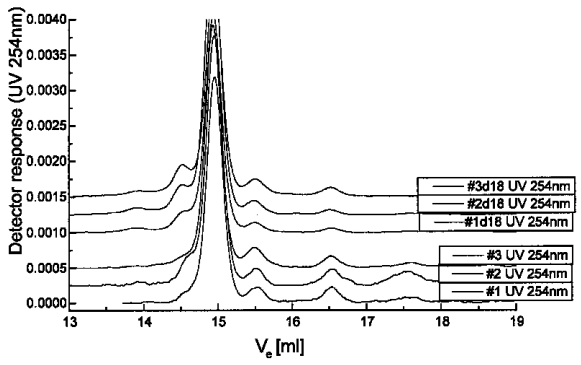

Figure 4 *GPC-UV (254 nm) comparison of original samples (lower elution profile) with samples stored in the dark for 18 months (upper elution profile). Eluent: THF, 1.00 mL/min. Injection volume: 20 µL. Sample concentration: 5 mg/mL eluent.*

Conclusion

A rapid and effective GPC method is described for monitoring the quality of virgin olive oil. In comparison to classical methods such as acidity value, peroxide value, and extinction coefficients K_{232} and K_{270}, the proposed method is more versatile. Dimerization and decomposition products can be detected sufficiently. The method provides a good fingerprint for determining authenticity and the presence of new constituents. The findings of this study also stress the need for good storage practices if the quality of virgin olive oil is to be maintained throughout its entire shelf-life.

References

1. Kiritsakis AK. Olive oil. From the tree to the table, 2nd ed. Food & Nutrition Press, 1998.
2. Aparicio R. Authentication. In: Warwood J, Aparicio R, eds. Handbook of olive oil, analysis and properties. Aspen Publishers, Inc., 2000:491–513.
3. Commission of the Codex Alimentarious. Standard for olive oils, 1993. CL 1993/15-FO.
4. Commission of the European Communities. Regulation no. 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Off J Eur Comm 1991, no. L248.
5. Fedeli E. Lipids of olives. In: Ralph E, Holman T, eds. Progress of chemistry of fats and other lipids. Paris, France: Pergamon Press, 1977:15–74.
6. International Union of Pure and Applied Chemistry (IUPAC). Standard methods for the analysis of oils, fats, and derivatives, 7th ed. Paquot C, Haufenna A, eds. Oxford, U.K.: Blackwell Scientific Publications, 1987.
7. Firestone D, Carson KL, Reina RJ. Update on control of olive oil adulteration and misbranding in the United States. J Am Oil Chem Soc 1988; 65:788–92.
8 El-Hamdy AH, El-Fizga NK. Detection of olive oil adulteration by measuring its authenticity factor using reversed-phase high-performance liquid chromatography. J Chromatogr A 1995; 708:351–5.
9. Rojo JA, Perkins EG. Cyclic fatty acid monomer formation in frying fats. Determination and structural study. J Am Oil Chem Soc 1987; 64:414–21.
10. Gertz C, Klostermann S. A new analytical procedure to differentiate virgin or non-refined from refined vegetable fats and oils. Eur J Lip Sci Technol 2000; 329–36.
11. Dauwe C, Reinhold G, Gertz C. Qualitative chromatographic analysis of oils and fats (in German). Chemie in Labor und Biotechnik 2000; 51:456–9.
12. German Standard Methods. Established by German Association of Fat Science. Standard method CIII 3d. Stuttgart, Germany: Scientific Publishing Ltd., 2000.

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