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Analysis and characterization of extra virgin olive oils

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Abstract

Samples of extra virgin olive oils obtained from the olive trees of the territory of San Giovanni in Fiore (CS), Italy, and common brands samples of extra virgin olive oils sold on the national territory, have been analysed according to EU regulation n. 61/2011 of the Commission of 24 January 2011. The analysis involved a high number of students in Calabria, Italy, who conducted with their teachers not only the experimental activity but also the elaboration of results and the writing of a scientific report. The examined extra virgin oils were characterized by investigating their acidity, the number of peroxides, the rancidity, and the spectrophotometric parameters that provide useful elements for assessing the composition and the quality. Furthermore, the infrared analysis provides useful data that help carry out quality control.

Keywords

Extra virgin olive oils (EVOO), Ultraviolet-visible spectroscopy, Fourier Transform Infrared spectroscopy, acidity, rancidity, carotenoid, chlorophyll.

Introduction

Calabria is the second biggest oil producer in Italy, with an average oil production per year which amounts to about 150,000 tons of oil from 550,000 tons of olives. Both traditional and modern culture are practiced, the first especially in the more internal areas, the second by industrial businesses using intensive culture and mechanical procedures [1]. We define *Virgin* any oil extracted exclusively by mechanical means, without using any chemical substance and not mixed with oils of different kind, while an oil to be defined *Extra virgin* must have a flawless taste and fully respect a number of chemical and physical parameters, among which the degree of free acidity (expressed in percentage/weight of oleic acid) is one of the most important.

The quality of an olive oil depends on a number of factors. Some of these factors are not modifiable because they belong to a specific geographical area (climate, terrain), others can be easily adapted or imitated (cultivation or extraction techniques). In any case oil extraction takes place by several procedures, but it is possible to affirm, on an empirical basis, that using either the classical or modern methods does not result in significant differences in the values of parameters determining the quality of the oil produced (acidity, number of peroxides, spectrophotometric absorption and organoleptic evaluation), [2].

The project on the quality of extra virgin olive oils involved 12 students attending the fifth year of the course specializing in Chemistry and Materials, age between 17 and 19, and lasted for three months. The students have been assisted by their teachers of Analytical Chemistry, Chemical Industrial Technologies and English throughout the field investigation, the lab experiments and analyses and the writing of a scientific report. The project is potentially the starting point of a network cooperation among members of the local community, which can eventually develop in further experiences and practices in the future.

Methodology

In our study the learning model chosen to design the present activity is Inquiry-Based Laboratory (ILAB), considered particularly suitable to enhance creativity in high school students, in accordance with the results of the study by I. Rahmawati, H. Sholichin and M. Arifin, [3].

Chemistry learning with ILAB requires a pre-designed worksheet to guide students during the activities. Students are required to solve a real - life problem, which in this case is to characterise and analyse extra virgin olive oils. The project has been designed combining CLIL methodology and Inquiry-based approach. The tasks and activities of the module focus on contents belonging to Non-Linguistic Subjects (Chemistry and Biochemistry), but at the same time aim at improving the students' receptive and productive skills in English according to the descriptors of the Common European Framework of Reference.

Table 1 shows the design of ILAB worksheet for this type of report.

ILAB Activity	Task	Activity	Purpose
1. Problem identification	Quality control of extra virgin olive oils	Students are faced with a real-life problem. In this activity the student must determine the main chemical-physical characteristics of extra virgin olive oils	Elicit ideas about acidity, oxidation reactions, pigments, rancidity.
2. Formulating hypotheses	Chemical-physical characterization of extra virgin olive oils (EVOO)	Students are asked to explain **Case#1**: What are the quality parameters that defines the chemical-physical and organoleptic characteristics of olive oils and olive pomace oils? Establish the methods for evaluating these characteristics. **Case#2**: How do we classify oils according to the acidity measured? **Case#3**: Why do high temperatures, exposure to light and oxygen damage the quality of olive oils? **Case#4**: Why does a low number of peroxides not necessarily imply a high quality? What kind of analysis must be performed? **Case#5**: Why is it important to perform the UV/Vis and FT-IR spectrophotometric analysis?	To determine which basic principles will be used and how they will be connected; To make hypotheses on the effects of environmental factors on the quality of olive oils; to perform creative thinking skills; To choose and practice the necessary technical procedures for each specific purpose; To research and organise the parameters for the quality characterisation of oils;
3. Design experiments	Acid-base titration Redox titration Spectrometric Qualitative analysis	Students design experiments procedure by determining control variables, independent variables and their own dependent variables	Apply the principles and knowledge previously acquired to verify the hypotheses previously formulated.
4. Collecting data	Titrations results Spectroscopic data	Students collect data from their designed experiment, as well as information needed to test the hypothesis.	Practicing volumetric analysis, determination of spectroscopic parameters
5. Data analysis	Interpretation of data	Students organize and analyse the data, Connect them to the hypothesis, make predictions, select which Findings are consistent with the information already possessed.	Developing and applying high-order thinking skills (analysing, evaluating, creating);

6. Conclusion	Quality control of extra-virgin olive oil	Students describe and interpret the findings obtained based on the results of hypothesis test.	Production of a written report with graphic representation of the findings.

Chemical Characteristics of Olive Oils

The oil obtained through any procedures utilized is a liquid lipid at room temperature. It is a non-polar organic compound, insoluble in water, but soluble in organic solvent. It is formed by 98%-99% of triglycerides (saponificable fraction) and a remaining 2% - 1% of an unsaponificable part. Depending on the chemical bonds present in the hydrocarbon chains, fatty acids are classified as saturated (when there are only sigma bonds) or unsaturated (with two or more double bonds).

Acidity

The acidity level of an oil (or a fatty substance in general) expresses the percentage content of free fatty acids. This parameter can be expressed in different ways, depending on the dominant fatty acid in the particular type of fatty substance. As a reference, we used the percentage of oleic acid present in 5 gr of substance. The free acidity content of the extra virgin olive oil, expressed as oleic acid, should not exceed 0,8 g per 100 g of oil.

Rancidity

The rancidity is expressed through the determination of

1. Number of peroxides,
2. Lea index
3. Kreis index

1. Number of peroxides

The number of peroxides is determined by the quantity of substances present in the sample, expressed in mill equivalents of active oxygen per Kg, which oxidize the potassium iodide. With this analysis it is possible to determine the degree of rancidity of the oil.

The number of peroxides must be less than 10 in olive oil in an excellent state of conservation, between 10 and 15 in good condition, less than 10 in refined olive oil and more than 20 in rancid olive oils. The analysis is carried out following the EC Regulation guidelines.

2. Lea index

The Lea index is indicated by the quantity of oxygen (in µg) present in 1 gr of oil and it expresses the state of rancidity of the oils: a Lea index of more than 100 is a sign of rancidity in progress.

3. Kreis index

Among the various essays proposed, the Kreis index is the most reliable to detect the presence of rancidity. It is a qualitative reaction based on the fact that if peroxides or hydroperoxides have formed in oil (as a result of rancidity), consequently ketones and oxyacids have been formed; these compounds react with fluoroglucine solution, resulting in a typical coloration.

Spectrometric measurements

Absorbance measurements in the ultraviolet/visible (UV/Vis) and infrared (IR) regions were conducted.

The UV absorbance measurements were made using a Jasco 660 UV/vis spectrophotometer. The specific extinction (extinction coefficient) in iso-octane at different wavelengths, including 232, 266, 270, 274 nm, was determined. Then, the variation of the specific extinction (ΔK) was determined. The corresponding equations are, [4], as follows:

$$K_\lambda = E_\lambda / c \times s$$

Where, K_λ = Specific extinction at wavelength λ; E_λ = Extinction measured at wavelength λ; c = concentration of the solution in g/100mL; and s = thickness of the cuvette in cm.

$$\Delta K = K_m - \frac{K_{m-4} + K_{m+4}}{2}$$

where, K_m = specific extinction at wavelength m (270).

The extinction coefficients of seven different samples of Extra Virgin Olive Oil (EVOO) were measured.

Conversely, the measurements of the absorption of electromagnetic radiations in the visible range provide information on the kind of photosynthetic pigments that constitute the unsaponificable fraction of oil.

A commercial FT-IR Spectrometer (Bruker Optics) was used to collect the FTIR spectra with a resolution of 4 cm^{-1} at 24 scans. Each IR spectrum was realized by collecting data in the 4000-600 cm^{-1} spectral range. The spectra are conducted in transmittance and converted into absorbance. FT-IR analysis of an olive oil provides important information on the acid composition. The FTIR spectra are characterized by the evident peaks at 2800-3100 cm^{-1}, at 1700-1800 cm^{-1} and at 800-1500 cm^{-1}. The specific frequency of a given vibration mode depends on the specific arrangement of the carbonyl groups and of the lipid hydrocarbon backbone, while the intensity of the specific vibration mode depends on the relative concentration of the related functional group [4].

Through the different methods of volumetric and spectrophotometric analysis, olive oils were analyzed from a qualitative point of view. The analysis was conducted on samples of:

- extra virgin olive oils produced locally by the families of the students involved in the project;
- extra virgin olive oils available in supermarkets;
- extra virgin olive oils supplied by the oil mill of the Portaro brothers involved in the realization of the project.

Results

Observing the experimental data obtained on acidity, we found that all EVOO are in agreement with EEC European regulations, with acidity values lower than 0.8% varying from 0.28% to 0.45%. According to the EEC regulations, the number of peroxides present in the samples must vary from 0 ÷ 12 meq for 5 ÷ 2 gr of substances; the values we found vary from 1.58 to 1.96, index of absence of rancidity. The Lea index indicates that a value above 100 is a sign of rancidity. The values we have obtained vary from 47 to 80. The Kreis index, among the essays, is the most certain to detect the presence of rancidity. In all the samples analyzed, no color changes were observed, a sign of absence of rancidity. All data are reported in Tab. 1a.

The spectrophotometric examination of a fatty substance in the UV range provides useful elements for assessing the composition and quality, as well as highlighting the industrial treatments.

The spectra of the oils have been plotted on a single graph and the wavelengths are taken at the absorbances of 232/266/270 and 274, and the ΔK are calculated. Specific extinction coefficients for Extra Virgin Olive Oil samples are reported in Tab. 1b.

K232 measures the absorption of ultraviolet light at a wavelength of 232 nanometres and tells us if the structure of the oil is changed as a result of an oxidation. In particular, the absorption at 232 nm increases for the formation of conjugated dienes.

K270 measures the absorption of ultraviolet light at a wavelength of 270 nanometres. In this case, the secondary oxidation state can be verified, that is the possible transformations of the oil structure following the formation of conjugated trienes.

ΔK monitors the state of secondary oxidation of extra virgin olive oil.

Visible light absorption of EVOOs is associated with the pigments' content. The presence of chlorophylls and carotenoids greatly influences the olive oils colour [5], which is a very important sensory parameter evaluated by consumers. A recent methodology, proposed by Cayuela et al. [6], associates the absorbance measured at specific wavelengths in the visible region, namely the K470 and K670 indexes, to the amount of carotenoids and chlorophyll derivatives, respectively. Here only three of the examined EVOO oils, the EVOO Portaro brothers, are reported in fig. 2.

The characteristics of mid-infrared spectra for biological EVOO Portaro brothers are shown in Figure 3. It is compared with a sunflower oil to evaluate the differences. The differences between the different samples were clearly small and occurred only in limited regions of the spectra, especially in peak intensities at fingerprint regions (1500–800 cm^{-1}) and at 3050-2750 cm^{-1}. For example in the inset of fig. 3 the clear shift of the 3009 cm^{-1} band (sunflower oil), attributed to the C-H stretching vibration of cis-double bond, to the 3005 cm^{-1} band (EVOO) can be noticed [7].

Conclusion

In the present work we have proposed a type of laboratorial activity developed within the teaching of Analytical chemistry. The authors involved students by using the Inquiry based Scientific Education (IBSE) methodology in the characterization of extra virgin olive oil utilizing volumetric and spectrometric analysis. All oils tested meet the eligibility criteria established by the European Regulation No. 61/2011 to be considered extra virgin olive oils. The project is potentially the starting point of a network cooperation among members of the local community, which can eventually develop in further experiences and practices in the future. For this reason we have chosen a food matrix representative of the economy of southern Italy, arousing attention, participation and interest from other actors, communities, partners and stakeholders.

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