NMR

NMR analysis for authenticity and quality control of olive oils

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Innovation with Integrity

Introduction

Olive oil is a vegetable liquid fat solely obtained from ripened olives. Its consumption for culinary purposes and as a source of well-being dates back to ancient times and remains a highly valued food today. Nowadays olive trees are cultivated in dozens of countries, with a clear predominance in Mediterranean regions.

In 2022, the global olive oil market reached $7.97 billion and is predicted to reach a value of $12.78 billion by 2028, a growth rate of 6.05 percent.[1]

The market for olive oil is being driven by the growing demand for healthy and natural food products, as well as its increasing use in the cosmetics and pharmaceutical industries.

Tasty, nutritive and healthy

As a source of many bioactive components, like triglycerides with mono-unsaturated and poly-unsaturated (essential) fatty acids, antioxidants (like polyphenols), vitamins (especially alpha-tocopherol as primary vitamin E type), minerals and aroma-creating volatiles, olive oil is highly valued as a tasty, nutritive, and healthy food, and as indispensable ingredient of Mediterranean dishes. The levels of these components in olive oil depend on a number of factors: mainly the olive cultivar (also referred to as variety), the olive trees' age, oil production processes, soil type, climate and weather conditions, harvesting time, and last but not least the oils' storage conditions.

Authenticity and quality are key

As consumers become more aware of the food products they consume, they demand more information about the food's provenance, authenticity, and production methods, whether in fresh or processed products. According to the US International Food Information Council (IFIC) Foundation, there is an increased number of people wishing to learn more about the origins of their food and its entire journey from farm to fork.[2]

The International Olive Council (IOC)3 - an international intergovernmental organization – defines highest quality olive oils as those obtained "solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration".[4] In turn, such oils are sub-classified by certain physico-chemical parameters and sensory criteria, where extra-virgin olive oil ("EVOO") is the superior olive oil category with the highest quality standard and commercial value.[4]

Combating olive oil fraud

Olive oil ever was – and still is – on the top-ten list of adulterated foods.[5] According to a report commissioned as part of the European Commission's Oleum Program,[6] the most common fraudulent practices or non-compliances to trade standards are mislabeling of lower olive oil qualities as extra-virgin olive oil, marketing blends of other - cheaper - vegetable oils (like sunflower oil) as olive oil, dilution with deodorized or low-quality olive oil, as well as mislabeling of the geographical or botanical origin.

To detect economically motivated adulteration of extra virgin olive oil obviously is of major importance to protect consumers' interests as well as producers' reputation and preserve trust in industry and governmental bodies.

The analytical challenge

Edible oils are complex mixtures of components, varying widely in concentrations as well as in chemical constitutions. But nothing is just random! In fact, all those components are metabolites of the respective oil-producing plants, and so each oil type - like rapeseed, sunflower, or olive oil – has its characteristic components' pattern.

Looking now into the type "olive oil", the different olive oil cultivars, the trees' geographical locations, and weather influences modulate the metabolism, and thereby introduce further variances to the olive oil components' pattern, typical for certain olive oil cultivars, countries of origin, harvest period, and further.

As being a natural product, certain metabolism continues after harvesting, and also production practices and storage conditions leave marks in the corresponding olive oil batches and unveil information about their quality.

And finally looking at single olive oils: there never will be two oils fully identical in all aspects: as fingerprints are unique to human individuals, each olive oil sample has its unique characteristics.

Olive oil producers, bottlers, exporters, buyers, and testing laboratories (commercial and governmental) likewise have strong interest in keeping control over the olive oils' authenticity and quality along the supply chains. These demands come along with analytical tools not only being capable to trace geographical origin and quality, but also quantify single oil components - and do this ideally without much sample preparation, and without altering the oils' individual matrix to maintain subtle information. In other words: an easy, quick, and reliable analytical solution to cover the screening range from whole matrices down to single components within one run.

Entering NMR spectroscopy

Nuclear Magnetic Resonance (NMR) is a non-destructive technique, using the inherent magnetic properties of specific atomic nuclei to characterize the molecular structure of a sample in solid-state or solution. It originally was predominantly used for the structure elucidation of single molecules, but quickly evolved into many other fields, like monitoring mixture compositions, study molecular dynamics (diffusion), interactions, or reaction kinetics, and quantify known and unknown components also in complex mixtures (like food) without the need of component separation.

600 microliters of a sample, for example an olive oil diluted in a solvent, is filled into a thin-walled "NMR-tube" which then is placed inside a powerful magnet. The magnet forces NMR-active nuclei (tiny "magnets" themselves) within the samples' molecules to align in the magnetic field. To observe for example the hydrogens (then called "Proton-NMR" or "^{1}H-NMR"), one or a sequence of short radio-frequency (rf) pulses emitted from the coil and tuned to the ^{1}H resonance frequency, perturbs the alignment for the duration of the rf-pulse impact. When the rf-pulse is switched off, a relaxing back to alignment starts, a tiny energy amount is released which is detected as a kind of "answer" (the free induction decay = FID) of the protons in the sample. The FID can be converted into an NMR spectrum by applying a mathematical algorithm called Fourier transformation.

To analyze NMR spectra, different paths can be followed, depending on the complexity of spectra and the type of analytical problem addressed:

If structure verification or elucidation of single components is needed, usually an NMR expert gets to work - nowadays often supported by software tools to automate routine steps and output possible structures.

In contrast, if a descriptive and/or predictive analysis of underlying relationships of components in complex mixtures is the goal - as for olive oil profiling - modern chemometrical (statistical) routines are applied. The focus here is not the identification of single components, but seeking for features or relations in olive oil spectra which are so-called classifiers for a certain geographical origin, or a quality like EVOO.

NMR spectroscopy has several strengths: it is intrinsically quantitative (the area of a certain signal directly corresponds to the number of protons contributing to this signal), highly reproducible, and capable to detect and quantify components covering a wide concentration range. Only one single quantification reference sample for all components in all samples, once prepared and re-usable for two years as daily standard, is needed. As far as sample preparation is done in strict accordance with the corresponding Standard Operation Procedure (SOP), and the NMR spectrometer is operating within predefined specifications, NMR data can be compared from lab-to-lab, worldwide and over time.

Last, but not least, NMR spectroscopy is both targeted and untargeted, allowing components to be detected which were not searched for.

Bruker's Olive Oil Profiling 2.0 solution

The Olive Oil Profiling 2.0 provides a solution for the authenticity and quality analysis of olive oils.

Uniquely within Bruker's current profiling solution portfolio, it is offered on two platforms: on the well-established 400 MHz FoodScreener™ instrument, and on the cryogen-free Fourier 80™ benchtop system. Both are conceptually identical: designed to be used in full automation, from sample setup via a LIMS-type software (SampleTrack™) to experimental runs and finally to analysis report generation. The two platforms solution addresses the different requirements of the olive oil industry, commercial testing laboratories and government laboratories.

The Olive Oil Profiling 2.0 solution's heart is a database of several thousands of NMR spectra of authentic olive oils, together with corresponding metadata, like geographical origin, oil quality, and quantified values of relevant olive oil authenticity and quality parameters (like free acidity, peroxide value, oleic acid content, total polyphenols, and more).

This database hosts all olive-oil-related variables to apply multiple machine learning techniques, with the goal to generate the models for countries, qualities, and parameters to be quantified.

So-called classification models identify and analyze joint behavior (“classifiers”) in NMR spectra which is unique for olive oil from a certain country, while taking into consideration that cultivar diversity, agronomical and environmental conditions, and other factors will add variation.

The same concept is applied to build olive oil quality models, like the one for extra virgin olive oil.

Another statistical approach to analyze data is the application of regression models to quantify olive oil parameters - without the necessity to identify their signals in the spectra, or even know their chemical structures. They base on correlations of (even subtle) NMR-spectral properties with corresponding values quantified by reference testing methods, like wet chemical analysis or chromatography (“regression metadata”). Such correlations are used to generate regression models for each parameter to be quantified.

Appropriate database management and profound validation ensures that such models are representative for the feature to be classified and are robust against natural variation and any further minor but inevitable data variations.

The Olive Oil Profiling 2.0 solution includes

- Verification of the country of origin (Greece, Italy and Spain)
- Quantification of 15 IOC regulated olive oil parameters, plus 5 additional parameters
- Verification of the quality grade Extra Virgin Olive Oil
- Detection of atypical olive oil profiles.

The time to result of the experimental run is 12 minutes (Fourier 80) and 25 minutes (FoodScreener), respectively.

For sample preparation, which is completed within a few minutes per sample, the olive oil is diluted in deuterated chloroform ($CDCl_3$), done by pipetting only. This mixture is ready to be filled into an 5mm NMR tube. The preparation is identical for the Fourier 80 and the FoodScreener instrumentation.

A user's perspective: The University of Seville

The University of Seville's Research, Technology, and Innovation Center (CITIUS) offers general research services to universities, public research organizations, and private companies.

Since the first NMR spectrometer was installed at the University of Seville in 1992, the Center has used Bruker's instruments in research applications. Now, CITIUS is using Bruker's FoodScreener with Olive Oil-Profiling to measure and evaluate olive oil samples with the objective of creating a reference database for use by the olive oil industry. Miguel Angel Rodríguez-Carvajal, Head of the NMR facility at CITIUS, commented on the use of the FoodScreener within the facility: "We are in collaboration with Bruker to measure olive oil samples, for which the FoodScreener has provided excellent results whilst providing more detailed analytical data than other methods – in a faster timeframe."

The FoodScreener provides the reproducibility and sensitivity for small sample analysis, with each sample requiring 214 microliters of oil. Manuel Angulo, NMR technician at CITIUS, added:

> *"Our focus is on acquiring NMR spectra for olive oil samples and the FoodScreener has proven to be a very powerful method. Instrument set up was straightforward thanks to Bruker's technical expertise."*

Find out more about Bruker's Olive Oil Profiling 2.0 solution
https://www.bruker.com/en/products-and-solutions/mr/nmr-food-solutions/olive-oil-profiling-nmr.html

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