ENZYMATIC vs FTNIR METHOD COMPARISON

The most widespread analysis systems in oenology are certainly the enzymatic and the NIR (or better, FTNIR). There are many supporters of one or the other system, which certainly have pros and cons, but to compare them it is necessary to know how they work evaluating their characteristics and limitations.

Principle of operation of enzymatic analyzers.

Enzymatic analyzers base their operation on the Lambert-Beer Law (the absorbance (A) measured at a given wavelength (λ) is linearly proportional to the concentration (C) of the substance and on the length of the optical path (a) through a constant of specific proportionality for each substance called *molar extinction coefficient* (ε_λ): $A = C \cdot in \cdot \varepsilon_\lambda$). If we had a pure solution of a substance with known ε_λ, it would be sufficient to measure the absorbance of the solution to determine the concentration of the substance. However, all the samples that are analyzed, both in the oenological field and in other areas, are very complex solutions and the absorbance is the sum of all the contributions of the substances present in the sample.

However, there are substances that react specifically with individual molecules, and only with them (as happens, for example, in an enzymatic reaction, where the enzyme is a specific catalyst for a given reaction). Even through one or more cascaded chemical reactions, new molecules can emerge that are distinguished from the rest of the sample by their high absorption at specific wavelengths. Therefore, it will be possible through these reactions to obtain a solution in which to measure the absorbance (or the variation of absorbance against time) that will be proportional exclusively to the analyte sought.

Enzymatic systems, such as DIONYSOS, are made up of a photometer, which measures absorbances, and of specific reagents for each analyte. Simply add the sample to the reagents to obtain a compound whose absorbance measured at a given wavelength is proportional to the concentration of analyte under consideration. This proportionality allows you to construct calibration lines from reference materials of known concentration (calibrator), which can later be used to deduce the concentration of the analyte in the sample.

FT-NIR systems working principle

The interaction of light matter is also the basis of these systems, but less energetic (infrared) wavelengths are used compared to enzymatic (UV-Visible) methods.

Infrared electromagnetic radiation vibrates the bonds of organic molecules, so that different functional groups within the individual molecule (eg -NH2 or -COOH) are excited by different frequencies. If we scan the absorbance of a pure substance in all the wavelengths included in a given range, we will obtain a characteristic graph of the substance (spectrum). If we compare the spectrum of a sample containing multiple substances, it is possible to identify them by comparing the spectrum obtained with those of pure substances and, again through comparison, to determine their concentration.

However, the spectra obtained with the classic IR spectrophotometers do not offer enough technical characteristics to be able to carry out a quantitative analysis. A more modern technique, called the Near Infrared Fourier Transform (FT-NIR), solves many of the problems of classical IR spectrophotometry: it has extremely fast times, a high signal-to-noise ratio, and a much higher resolution, which allows a better distinction of the various organic functional groups.

FT-NIR spectrometers produce a signal, called an interferogram, that contains the absorbances at all analyzed frequencies encoded within it. It is produced through a light signal that is split into two halves; one half will have a fixed

length, while the other will vary in length thanks to a movable mirror. When the two signals recombine, they will produce positive or negative interferences according to the different lengths. This new signal is passed through the sample and then to a detector. The signal can be measured very quickly, performing several scans per second. The interferogram is then processed through a mathematical process called the Fourier transform, which gives rise to the spectrum of the sample under examination. At this point, the sample spectrum is compared to a database of hundreds of different spectra from various samples in which the components of interest have been determined. The concentration of the test sample is statistically estimated by comparing it with the database. In other words, the results obtained are not actually measured, but are obtained through statistical analysis.

Pros and cons

Many of the enzymatic methods have been included among the official methods of the OIV, while the FT-NIR, which cannot be calibrated with reference standards such as enzymatic systems, does not meet the requirements to become a reference as it is not properly a measurement method. This does not mean that the FT-NIR methods are not valid, but that they cannot be used to issue certified values.

FT-NIR systems are extremely faster and deliver a set of results in just a few seconds. Enzyme systems are slower and require incubation times of the order of 5-10 minutes to complete reactions and proceed with measurements.

Enzymatic systems are "open" in nature and the entire measurement process is visible and monitored; the various methods must be calibrated and controlled, which requires a certain participation of the operator who can intervene at any time to correct problems (turbidity of the samples, for example). In contrast, FT-NIR systems are “closed black boxes” in which it is not possible to intervene in the measurement, correct the calibration or detect interference that modifies the optical signal. The signal processing and the calculation of the results is entrusted to the software, which in turn relies on a database that is useful only to the extent that the sample is perfectly represented in the data set used.

Enzymatic methods have much wider measurement ranges than FT-NIR, and are much more sensitive. In fact, the FT-NIR is unable to adequately determine concentrations below 0.2 g/L, approximately since the signal-to-noise ratio is very low. Adequate sensitivity is relevant for the winemaker in certain processes, such as the end of alcoholic fermentation or the beginning of malolactic fermentation, in which it is necessary to recognize extremely small amounts of analyte and identify the right moment to act.

Enzyme reagents are highly specific, so that they only recognize the molecule of interest, being able to distinguish between chiral molecules. For example, it is possible to distinguish between D- and L- stereoisomers, whereas with FT-NIR you will always obtain only the sum of the two since both molecules have the same chemical structure and bond arrangement.

Another important difference between the two systems is their scalability: an FT-NIR system offers simultaneous results for a defined set of analytes. Although FT-NIRs do not require consumables or reagents, this set of analytes cannot be modified or reduced, but cannot be expanded either, since the result is generated from a mathematical function that incorporates them as parameters; therefore, if the analyte is not included in the definition of said function (which in turn is generated by the database used), it cannot be added later.

With enzymatic systems, on the other hand, since they are composed of an instrument (manual or automatic photometer) and reagents (each reagent is specific for a single analyte), it is possible to choose which analytes to dose and not necessarily all of them must be used together. This leads to complete freedom in the use of the enzyme system, which is much more flexible for the specific and seasonal needs of the winery.

The FT-NIR systems exploit very advanced technologies and very powerful software, which determine a very high cost for instrumentation and, although little is required of the operator in terms of daily maintenance, they require periodic

maintenance by specialized personnel with high costs. On the other hand, enzyme systems, even automatic ones, take advantage of consolidated technology that has lower costs, both initial and maintenance.

Characteristic	FT-NIR	Enzymatic
Analysis	Statistical estimation of concentration against a database	Quantitative measurement of analytes by specific enzymatic reactions
Calibration	It is only possible to adjust the result by comparing with other analysis methods	Calibration with reference material
Use	Very easy to use: just push a button	Requires some user training
Monitoring	They are closed 'black boxes' that do not allow intervention	They are open systems that allow action
Cost	Very expensive, but do not require consumables or reagents	Economical, but require consumables and reagents
Time to results	Results in less than a minute	Results between 5 and 10 minutes
Maintenance	Little, but can only be done by the manufacturer due to its complexity (high cost)	Daily and simple maintenance by the user, and periodically by the manufacturer, of low complexity (low cost)
Sensitivity and accuracy	Limited	Very sensitive and reproducible methods
Scalability	Not possible	Total
Official OIV / AOAC	Not approved	Quite a few official OIV methods (not all)

Conclusions

Enzyme systems and FT-NIR are totally different systems and a direct comparison between them is not possible, except when we refer to specific process needs. Most often, the two systems are used together, amplifying the advantages and eliminating the disadvantages. In many warehouses, during periods of intense work, such as harvesting, FT-NIRs are used to process numerous samples in a short time, obtaining sufficient initial results for this purpose. Later, however, the use of enzyme systems allows the results to be verified with greater precision and accuracy and a greater guarantee of data security. Additionally, enzyme systems can be used as a simple, inexpensive, and safe method to verify and calibrate FT-NIR systems.

For more than 10 years, Sinatech's commitment to the winemaker has been working side by side to provide the most appropriate analytical solutions to the control and monitoring of the winemaking process. Automated methods easily adaptable to any work routine, with a personalized advisory team to help you quickly and smoothly implement.

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