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INDUSTRY 4.0 IN THE AGRI-FOOD SECTOR: INNOVATIVE SENSORS AND SMART LOGISTICS TO SUPPORT THE SUSTAINABILITY OF THE SUPPLY CHAIN

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N.B.:

AAM = Authors Accepted Manuscript

TBS = To Be Submitted

S = Submitted

1. ABSTRACT

The agri-food sector is undergoing significant transformations in measurement technologies, with traditional laboratory-based analytical techniques increasingly being replaced by rapid, non-destructive, and environmentally friendly methods. Visible and Near-Infrared (vis/NIR) spectroscopy and imaging techniques are highly regarded for their non-destructive sampling, real-time results, and ability to perform quality controls throughout the production process.
However, these techniques typically involve expensive, complex instruments often impossible to use outside of a specialized laboratory. To overcome these problems, low-cost prototypes are being created that maintain the reliability of laboratory instruments, although with significantly lower performance. The aim is to ensure high-quality products that meet customer demands for healthiness and sustainability through meticulous quality controls. Chemometrics plays a crucial role in interpreting data, predictive modeling, and variable selection, which can simplify these devices. This project aims to meet the needs of high value-added supply chains by implementing simplified spectroscopic devices utilizing a limited number of wavelengths.

Over the three years, not only were prototypes of portable instruments used, but several studies also involved the use of benchtop instruments. These served as a reference for the optical acquisitions obtained using the prototypes, as well as for conducting feasibility studies and with the aim of implementing this type of instrumentation in-line within the food industry.
Commercial instruments used included Aurora NIR, (Grainit), ASD Quality SpecTrek, Jaz (Ocean Optics), Corona Process® (Zeiss), and Unity® (Hellma). Data analysis followed a specific procedure involving spectra exploration, chemometrics analyses and modeling, using Microsoft Excel, Matlab® and the PLSToolbox package as the main software.

A first prototype, which also led to the filing of a patent, was developed and tested on olives, tomatoes and wine grapes and it has been designed specifically for use on small-sized matrices. The optical device was used to evaluate table tomatoes' quality features and then to quantify qualitative parameters of Chardonnay grapes directly in the field. Regarding wine grapes, Life Cycle Assessment (LCA) methodology was also applied to evaluate the sustainability of the optical analysis compared to traditional laboratory analyses, demonstrating the environmental advantages of spectroscopy.

Research activities continued with grape matrices during post-harvest phases, using benchtop instruments to measure key parameters at consignment. The feasibility of vis/NIR spectroscopy for rapid polyphenol content determination in grapes was assessed and the final models provided valuable information on phenolic parameters, with promising results that require further testing in wineries.

Another experiment focused on evaluating the environmental and economic performance of an automated system for managing yeast nutrition and monitoring the fermentation process. In line with the principles of 4.0 winemaking, another simplified prototype was developed with the aim of analyzing non-solid matrices, such as wine, must, or other liquids, for detecting adulterations and measuring qualitative parameters. This prototype was used to monitor wine must fermentation and craft beer production, testing its applicability to different liquid matrices and fermentation types.

Another experiment was conducted using benchtop instruments for the determination of acrylamide in cocoa biscuits, yielding promising results that align perfectly with Industry 4.0 and the in-line implementation of spectrophotometric analyses.

A third prototype has been developed and tested but is still in the pre-prototype stage (Technology Readiness Level, TRL of 3 out of 9) and requires further experimentation before reaching the performance of the other two prototypes (TRL 4). Experiments were conducted on larger agri-food matrices, such as apples and pears, always using commercial instruments as references, given the early stage of development of the prototype. The goal was to monitor qualitative parameters and create predictive models for ripening and senescence.

Visible/Near-Infrared spectroscopy is a valuable technology for rapidly, non-destructively, and sustainably assessing quality parameters of several food and agricultural products. The prototypes developed showed promise for certain applications but require further experimentation. Further development and testing are necessary to enhance the applicability of these prototypes in the agri-food sector.

2. INTRODUCTION AND LITERATURE REVIEW

2.1 INDUSTRY 4.0

According to a widely held view among economic historians, modern times have experienced three significant technological shifts. The first began with the 18th-century industrial revolution, driven by the introduction of mechanization, steam power and waterpower. The second industrial revolution was characterized by the use of the internal combustion engine and electric motor, which enabled the electrification of factories and homes, as well as the postwar expansion of automotive infrastructure. The third industrial revolution emerged with the development of electronics and the Internet (Taalbi, 2019).

This progression led to the fourth industrial revolution, also known as Industry 4.0 or I4.0, characterized by the rapid technological advancements of the 21st century or the integration of various digital technologies into manufacturing and industrial practices. Traditional understanding attributes the "invention" of Industry 4.0 to 2011 in Germany, when a working group, under the auspices of the Research Union Economy Science of the German Ministry of Education and Research, introduced the concept at the Hanover Fair (Kagermann et al., 2013, Marzano & Martinovs, 2020). According to other sources, the term was coined in 2016 and can be attributed to Klaus Schwab (Schwab, 2017), the founder and executive chairman of the World Economic Forum, and it signifies a major transformation in industrial capitalism.

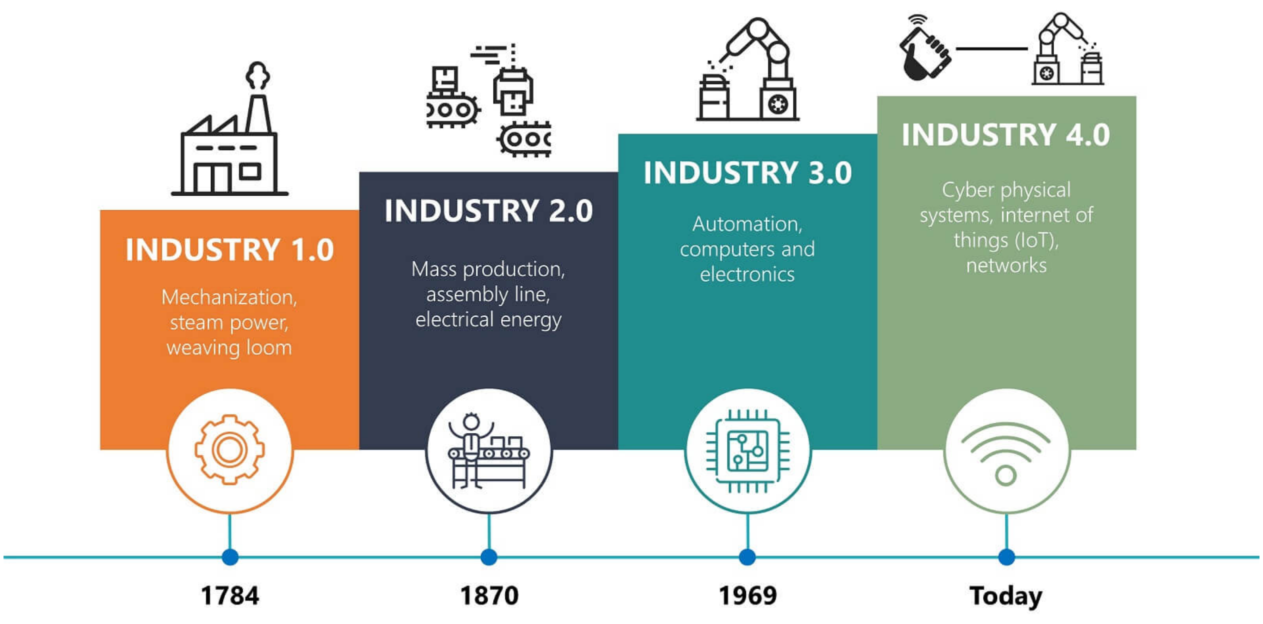

Figure 2.1.1 The progression of the industrial revolution from the first to the fourth.

I4.0 integrates machines, sensors, and various devices with personnel overseeing production processes and efficiency via wireless networking technologies, IoT capabilities and Artificial Intelligence (AI). This enhanced connectivity allows operators throughout the production chain of different sectors to collect large datasets and specialized knowledge, facilitating development and identifying key areas for innovation and transformation (Javaid et al., 2022).

There remains considerable disagreement and misalignment regarding the technologies that constitute Industry 4.0. These technologies are often described in relation to specific applications concerning various production operations, which is why the specific functioning of a technology, and its performance may vary (Chiarello et al., 2018).

There are several technologies that can be attributed to I4.0: Internet of Things (IoT), Big Data, Cloud Computing, Additive Manufacturing, Autonomous Robots, System Integration, Augmented Reality (AR), Cyber-Physical Systems (CPSs), electric vehicles, and Simulation (Bigliardi et al. 2020).

Industry 4.0, in general, is driven by technological developments such as service-oriented architectures, which enable the creation of various service utilities. These concepts converge into what is known as smart production or smart manufacturing, characterized by the creation of personalized products and a very high level of collaboration through production networks. The logistics, agri-food, and automotive sectors are among those that can be primarily and positively affected by Industry 4.0 (Müller, 2019).

2.2 INDUSTRY 4.0 IN THE AGRI-FOOD SECTOR

The food industry is continuously evolving, with technology playing a crucial role. Scientific and technical advancements allow for the production of food and beverages that better meet consumer demands safely, utilizing more sustainable and efficient production processes to meet the needs of global markets (Luque et al., 2017).

In this sector the human operator still plays a very important role, therefore, Industry 4.0 could be further exploited. There are various applications in the agri-food sector that would benefit from the innovative technologies of I4.0, such as monitoring, automation, and decision support (Oltra-Mestre et al., 2021).

According to Leal Filho (2022) another important aspect to consider is that while the food industry is one of the major contributors to climate change, food production must be increased to meet the growing demand of the ever-expanding population. Many food industries are striving to adopt sustainable and innovative technologies to achieve high standards of efficiency and performance. The potential of these technologies and digital solutions is becoming increasingly recognized, and in the post-COVID-19 era, the adoption of digital technologies throughout the entire food supply chain has accelerated further (Hassoun et al., 2023).

The global population is projected to continue growing, reaching approximately 10 billion by 2050. It has been estimated that major cereal crops (wheat, rice, and maize) will decline due to the current situation regarding greenhouse gas emissions and ongoing climate change. According to Arora (2019), the disproportionate increase in population and climate change can be identified as the primary threats to agricultural production and meeting global food demand. It is imperative that research continues towards intelligent agricultural solutions that optimize the use of increasingly limited resources. Therefore, a reduction in food waste, efficient use of water and energy, and continuous monitoring of available resources are essential (Senturk et al., 2023).

It should be noted that the complexity of agri-food systems is significantly high due to the involvement of numerous unpredictable variables in agriculture, the diversity of food materials, and the varied food habits of consumers. This complexity makes it challenging to convert the knowledge of farmers, industry experts, and consumers into clearly defined rules (computer programs) that can be implemented in AI-based (Artificial Intelligence) expert systems (Goyache et al., 2001) which could however contribute to generate a vast array of datasets that vary in content, structure, and storage format through the use of different IoT devices. Managing and storing this extensive data necessitates sophisticated methods, along with advanced statistical techniques and programming models to extract meaningful insights (Lokers et al., 2016).

Technologies that facilitate reduced use of agricultural resources such as water, fertilizers, and agrochemicals, and significantly lower the carbon footprint of farming, will be crucial for achieving global agricultural sustainability. Similarly, eco-friendly strategies to protect food crops or products from decay and pests, thereby reducing losses and extending shelf life, are essential to tackle global food security challenges. The implementation of these modern technologies in agriculture is commonly known as “smart farming” or “precision agriculture” (Misra et al., 2020).

Big Data technologies are crucial in this context: machines are equipped with various sensors that collect environmental data used to inform their operation. This ranges from relatively simple feedback mechanisms, like temperature control, to deep learning algorithms for tasks such as determining the optimal crop protection strategy. Additionally, these systems integrate data from external Big Data sources, such as weather forecasts, market trends, or benchmarks from other farms, to enhance their effectiveness (Wolfert et al., 2017).

With regard to the supply chain and traceability, it is evident that this represents another particularly challenging aspect due to the necessity for advanced control systems to manage perishables, fluctuating supply-demand variations, and stringent food safety and sustainability goals. The utilization of IoT networks incorporating sensors for humidity, temperature, light, microbiological, and product quality for real-time monitoring of goods in transit proves invaluable for the food industry, enabling rescheduling, recalls, or other appropriate actions (Lezoche et al., 2020). Despite the anticipated benefits, the integration of IoT with business processes remains in the very early stages of development within food supply chains and the food industry as a whole.

Another aspect to consider is that consumer awareness regarding food composition and quality has significantly increased due to a growing emphasis on healthy lifestyles and advancements in food science and technology. Additionally, food safety regulations now require detailed labeling of product composition and rigorous quality monitoring. In this context, IoT sensors and Big Data based on UV-Visible-Near Infrared Spectroscopy (UV-Vis-NIRS) are emerging as key tools in the assessment of food composition, quality, and safety (Pandiselvam et al., 2022).

2.3 OPTICAL SENSING IN THE AGRICULTURE 4.0 SECTOR

The objective of the book chapter below is to give a comprehensive overview of vis/NIR spectroscopy and other optical analysis applied in the agriculture sector. Some basic principles of techniques and multivariate statistical analysis, which are essential to apply to this type of data, are also explained. The chapter then focuses on a specific application of these techniques to the olive oil industry and the immense potential represented by portable instruments. It also introduces the experimentation that led to the filing of a patent for a portable spectrophotometer, which will be further explored in two papers in the results section of this thesis work (chapter 4.1).

BOOK CHAPTER: Optimization of the Olive Production Chain through Optical Techniques and Development of New Cost-Effective Optical Systems Inspired by Agriculture 4.0

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Abstract

Industry 4.0 is characterized by autonomous decision-making processes, monitoring assets and processes in real time and to real-time connected networks through early involvement of stakeholders. In this scenario, there is a growing interest and a need of innovation also in the agri-food system in the production processes and quality control through the development of new interconnected sensors (IoT approach). Hardware minimization, as well as software minimization and ease of integration, is essential to obtain feasible robotic systems. A substantial change in measurement methodologies is therefore ongoing, and it is of interest the opportunity to replace the consolidated analytical techniques, based on laboratory analyses, with methods based mainly on physical approaches of rapid execution, of limited invasiveness, and with high environmental sustainability. These approaches should be applicable directly in the field or in operative environment, allowing the creation of big databases characterizing the samples, particularly large and shared through the data cloud. This chapter will aim to overview the theoretical principles of the most important technologies applied to the olive oil sector presenting some case studies and will be focused on the future perspective for all operators of the olive sector who want to use a sustainable approach and olive-growing 4.0.

Keywords: agriculture 4.0, optical analysis, Vis/NIR spectroscopy, chemometrics, sensors, qualitative parameters, green technology, machine learning, simplified system

1. Introduction

The agri-food system is increasingly showing the need to innovate production processes and the related quality controls through the use of new technologies and the use of innovative sensors that could be interconnected, approaching what is called industry 4.0. In this context, and in particular in agriculture 4.0, emerging technologies such as artificial intelligence, big data, Internet of Things (IoT) are presented as a solution to the new challenges associated with food production. It is a digitization of all agricultural systems capable of increasing yields by reducing inputs and labor requirements. Furthermore, these technologies are capable of improving the health of the environment by enabling the production of a higher amount of food on the existing land while saving further land conversions and increasing eco-efficiency [1]. Obtaining high-quality and safe agricultural and food products is now an essential condition for both producers and consumers who are more involved and interested in the various aspects concerning food production. Therefore, the agri-food industry is currently concentrating on the production of healthy products that at the same time meet the market demands, and to do this it is essential to carry out punctual and precise quality controls on the products [2].

The analytical methods currently available to assess quality require time and above all are destructive techniques or laboratory chemical analyses that also involve the use of reagents. Nondestructive techniques based on optical properties and visual evaluations of food matrices are now being used all over the world as a response to these needs. One of the most widespread techniques is undoubtedly visible and near-infrared (vis/NIR) spectroscopy, which is based on the measurement of the variation in the spectral characteristics of a sample irradiated with electromagnetic radiation in the visible and in the near-infrared range (400–2500 nm). The variations of the spectral characteristics in a matrix can be recorded in different modalities according to the characteristics of the product but also according to the characteristics of the instruments used. Spectroscopy for analyzing agricultural and food products has proven to be an exceptional and rapid tool with little or no sample preparation [3].

This type of nondestructive technique guarantees the reduction and, in some cases, even the elimination of the use of solvents, which are instead necessary to carry out traditional chemical laboratory analyzes. Compared with vis/NIR technology, chemical techniques require a lot of time, sample preparation, and the use of chemical reagents influencing both the cost aspect and the

environmental impact aspects. Moreover, in recent years, research tends to pay attention also to on/in/at-line applications, and vis/NIR spectroscopy offers several opportunities for quality control during processes: the replacement of the analytical tools and reagents related to chemical analyses with one vis/NIR spectrometer could reduce the environmental impact of analyses [4].
Vis/NIR spectroscopy is just one example of the numerous techniques that are being implemented in these fields. Paragraph 2 of the chapter will analyze the principles of the most common nondestructive techniques used in the agri-food industry, paragraph 3 will focus on the applications of these techniques in the optimization of the olive production process, and finally, fourth paragraph will illustrate the portable prototypes and future prospects of simplified optical devices.

2. Main optical nondestructive approaches and data analysis

2.1 Vis/NIR and NIR spectroscopy

Among the nondestructive techniques, spectroscopic analyses in the visible–near infrared (vis/NIR) and near infrared (NIR) regions are widely used in different fields. Since the early 1970s, various instruments have been built that are able to exploit these technologies: instruments that acquire the sample spectrum in a specific wavelength range and record the average spectrum of a single defined area of a sample. Vis/NIR and NIR spectroscopies are used to acquire punctual information on the nature of the functional groups present in a molecule by exploiting the interaction between light and the structure of a sample. The electromagnetic radiation is in fact able to promote vibrational transitions in the molecules. Spectra in the visible region (between 400 and 700 nm) and spectra in the near infrared region (between 700 and 2500 nm) are composed of combination and overtone bands related to absorption frequencies in the mid-infrared region (MIR, between 2500 and 50,000 nm). All these combinations and overtone bands correspond to the frequencies of the vibrations between the bonds of the atoms that compose the molecules of the analyzed matrix. Each matrix or material is a unique seal of atoms so there are no two compounds capable of producing the same vis/NIR spectra.
Through the use of chemometric statistical analyses, it is possible to use spectroscopy as an excellent tool to perform quantitative analyses. A peculiar aspect of this technique is that it does not require sample preparation, thus offering a valid alternative to traditional chemical or physical analytical methods, which instead requires time and the use of solvents or other materials. The data deriving from the spectroscopic analyses are complex and require specific statistical analyses to obtain the information of interest [5].

2.1.1 Principles and instrumentation

The chemical composition and physical characteristics of a sample determine reflection, absorption, or transmission of the electromagnetic radiation. The reflected light could cause specular reflection shine (to be avoided), while diffuse reflection is produced by rough surfaces. These reflection phenomena provide information on the sample surface. More interesting could be the scattering resulting from multiple refractions within the material. The sample heterogeneity is highly influencing the scattering effects. Also, size, shape, and microstructure of the particles have an effect on scattering. Scattering affects the reflected spectrum, while the sample shape is more related to the absorption process. The bands of absorption in the NIR region are mainly overtones and combination bands of the fundamental absorption bands in the IR region, deriving from vibrational and/or rotational transitions. In the case of complex matrices such as foods, multiple bands and the effect of the widening of the peaks determine vis/NIR and NIR spectra with a wide coverage and few acute peaks.

To acquire a spectrum, it is necessary to use an instrument called a spectrophotometer, which consists of a light source, an accessory to present the sample, a monochromator, a detector, and optical components. Spectrophotometers are classified according to the type of monochromator: it is a device able to decompose a single polychromatic light beam into several monochromatic light beams (that contains waves of a single frequency), thus allowing to analyze the intensity as a function of wavelength. In a filter instrument, the monochromator is a wheel holding absorption or interference filters and has a limited spectral resolution. In a scanning monochromator instrument, a grating or a prism is used to separate the individual frequencies of the radiation entering or leaving the sample so the radiation at the different wavelengths can hit the detector.

Spectrophotometers based on Fourier transform use an interferometer to generate a modulated light beam. Using the Fourier transform, the light reflected or transmitted by the sample is converted into a spectrum. The most diffused systems use the Michelson interferometer, but also polarization interferometers are employed in the optical bench of some instruments. The photodiode array (PDA) spectrophotometers have a wide diffusion; these systems are based on a fixed grating, which focuses the radiation onto a silicon array of photodiode detectors. The systems based on laser do not use monochromator but different laser sources or a tunable laser. Finally, acoustic optic tunable filter (AOTF) and liquid crystal tunable filter (LCTF) instruments are available on the market. AOTF uses a diffraction-based optical-band-pass filter easily tunable varying the frequency of an acoustic wave propagating through an anisotropic crystal medium. LCTF instruments use a filter to create interference in phase between the ordinary and extraordinary light rays passing through a liquid crystal. The combination of different tunable stages in series can result in a high resolution.

2.2 Computer vision and image analysis

One of the limitations of spectroscopic analyses is the punctual measurement and therefore the inability to provide information on the distribution of an object. Depending on the uniformity of the qualitative attribute to measure, it may be necessary to repeat the spectral acquisition in several points on the sample. In order to get the spatial distribution, vision technique is a solution. With the huge development of imaging technology, computer vision results attracting for agri-food industry. A large number of applications have been developed for quality inspection, classification, and evaluation of agri-food products [6, 7]. Image data can reflect many external features of a sample such as color, shape, size, surface defects, or contaminations. Computer vision has been applied to solve various food engineering problems ranging from quality evaluation of foodstuffs to quality attributes unavailable to human evaluators. Computer vision tools are powerful but not much useful for in-depth investigation of internal characteristics. This is due to the very limited capability to provide spectral information with this technique.

2.2.1 Multispectral and hyperspectral images

RGB images, represented by three overlapping monochrome images, are the simplest example of multichannel images. The multispectral images are usually acquired in three/ten spectral bands including in the range of visible, but also in the range of infrared, fairly spaced. In this way it is possible to extract a larger amount of information from the images respect to those normally obtained from the RGB image analysis. The bands that are used in this analysis are the band of blue (430–490 nm), the band of green (491–560 nm), the band of red (620–700 nm), and the band of NIR and MIR. Different spectral combinations can be used depending on the research aims.
The combination of NIR-R-G (near infrared, red, green) is often used to identify green areas, for example, from satellite images. On the contrary, the combined use of NIR-R-B (near infrared, red, blue) is very useful to analyze fruit ripeness, thanks to chlorophyll absorption in the red range. Finally, the combination of NIR-MIR-blue (NIR, MIR, and blue) could be used to observe the sea and ocean depth. Hyperspectral imaging (HSI) is a powerful tool combining spectroscopy and imaging into a three-dimensional data structure (hypercube). The HSI is based on the acquisition of a large number of images at different spectral bands, allowing analysis of each pixel obtaining

at the same time a spectrum associated with it. The data structure of a hyperspectral image is data cube, considering two spatial directions and one spectral dimension.

Hyperspectral technology can integrate the advantages of conventional digital imaging and spectroscopy to obtain both spatial and spectral information from an object simultaneously.

In recent years, HSI has been applied to food safety and quality detection, because the technology can achieve rapid and nondestructive detection of food, and the requirement to experimental condition is low [8]. HSI has opened up new possibilities within agri-food analysis, in particular Liu et al. [9] outlined detailed applications in various food processes including cooking, drying, chilling, freezing and storage, and salt curing, emphasizing the ability of HSI technique to detect internal and external quality parameters in different food processes [9].

Using HSI, the hypercube can be acquired in reflectance, transmission, and fluorescence. Nevertheless, the most used acquisition techniques for spectral images are reflectance, transmission, and emission, considering the scientific works published. HSI has many advantages, e.g., the huge time savings that can be obtained for the application to industrial production processes. The advantages of HSI for the agri-food sector can be listed as follows: (i) not necessary sample preparation; (ii) noninvasive methodology that avoids sample losses; (iii) economic value related to time, labor, reagents, savings, and a strong cost-saving for waste treatment; (iv) for each pixel of the sample is acquired the full spectrum and not only few wavelengths; (v) many constituents can be predicted at the same time simultaneously; (vi) special region of interest could be selected and analyzed. The hypercube generated by using HSI provides a large dataset. The information derived from the hypercube may contain also redundant information. This data abundance may cause a high computational load due also to the long acquisition time. Therefore, it is desirable to reduce this load at acceptable levels, considering the application of HSI for real-time application. For this purpose, the spectral image is appropriately reduced using chemometric data processing, mainly selecting the most informative wavelengths. Using the selected spectral bands, a multispectral system can be envisaged for application at industrial level.

2.3 Chemometrics in agri-food sector

Chemometrics is defined as a branch of chemistry that studies the application of mathematical or statistical methods to chemical data. The International Chemometrics Society (ICS) defines it as a chemical discipline that uses mathematical and statistical methods to: design/select optimal procedures and experiments, provide maximum chemical information by analyzing data, give a graphical representation of this information, in other words, information aspects of chemistry. Chemometrics is essential for processing multivariate data obtained by optical techniques and for obtaining useful information for solving problems related to spectral noise.

One of the most used techniques is the Principal Component Analysis (PCA), also known as the Karhunen-Loève transform. It is an unsupervised exploratory qualitative analysis technique that allows reducing the more or less high number of variables describing a set of data to a smaller number of latent variables, limiting the loss of information.

Other chemometric techniques used extensively in these fields are supervised techniques, techniques that require method validation and that are used to obtain the quantitative prediction of the parameters of interest. Among these we find regression techniques such as Partial Least Square (PLS) regression or Multiple Linear Regression (MLR). The models developed using these techniques must then be tested using independent samples as validation sets to verify the accuracy and robustness of the model.

3. Application of nondestructive techniques for the optimization of the olive production process and enhancement of by-products

Agricultural products are converted into food products by using different processes. The process to achieve the best performance is carried out considering both efficiency and the target quality of the final food product, in order to be competitive on the market. The production of a high-quality extra virgin olive oil (EVOO) could be reached considering an optimization of the different production steps: olive harvesting and handling; milling operation to be done in a short time after harvesting; use of a modern milling plant equipped with suitable technologies to control process conditions. A high level of control of the standard operating conditions is a crucial aspect to avoid process failures and to maintain the highest final product's quality.

During the ripening process, the olives undergo the variation of various physical parameters such as weight, color, pulp-to-stone ratio, and texture and also of chemical parameters such as oil content, fatty acid composition and polyphenol, tocopherols, and sterols content. These characteristics are of great importance because they influence the quality, the yield, and the shelf-life of olive oil and of the by-products of olive production. Olive oils deriving from overripe fruits, for example, have a reduced shelf-life due to the increase in polyunsaturated fatty acids and the decrease in the total content of polyphenols. In particular, in the olive oil extraction chain, process control and management determine the conditions for producing high-quality oil, which is essential both to maintain consumer confidence and to evaluate potential plant yield losses. The flow sheet of the process is based on the following steps: olive cleaning, crushing to obtain a paste, paste malaxation, solid liquid separation, and liquids separation. Solid–liquid separation is a crucial aspect of the entire process. It is based on the separation of the solids (called pomace) from the other components, namely oil and wastewater. It is important to have online information on the oil content of the olives to set corrective actions during the process in order to reach the best extraction performance.

Nowadays the consolidated analysis protocol is based on the Soxhlet method to analyze the oil content in olives, pomace, and pate. This protocol requires a time consuming drying step, followed by an extraction based on the use of solvent. For this issue, the Soxhlet method is often substituted in routine analyses by Nuclear Magnetic Resonance (NMR) spectroscopy. Also, this procedure is not sufficiently fast due to water interference (the olive pomace must be completely dry). Consequently, this method is unsuitable for an online application. A precise monitoring of the intermediate products between the olives entering and the oil outlet (the paste, the pomace, and the pate) is crucial for control of the process progress. It is useful to establish correlations among olives, paste, pomace, patè, and oil. For this aim, rapid and possibly easy-to-use technologies are required to assess olive ripening and the characteristics of the by-products. In this way an early detection of possible failures and a continuous monitoring of the production process during its crucial steps result in an adequate control of the oil quality and yield. From this point of view, nondestructive optical applications could greatly help the sector.

Several studies have highlighted the enormous opportunities offered by NIR spectroscopy in terms of applications for quality control during the process, performing on/in/at-line measurements on olive fruits, on pastes, and on oils [10]. Researchers tend to focus attention on the online applications of noninvasive technologies in order to reduce the gap between laboratory scale experimentation and the olive milling industry [11]. A number of studies applying different vibrational techniques in the olive oil chain can be found in the literature, mainly with the aim of standardizing the procedure for an application as official control of the end product [12]. For this purpose, it is crucial to evaluate the optimal spectral range to be used, and the chemometric methods to be performed to obtain robust predictive models for the estimated parameters.

On intact olives, Beghi et al. [13] studied the capability of portable vis/NIR and NIR spectrophotometers to investigate different texture indices for the characterization of olive fruits entering the milling process. Salguero-Chaparro et al. [14] used NIR spectroscopy for the online determination of the oil content, moisture, and free acidity performing measurements directly on intact olives. NIR was used for the analysis of olive by-products (e.g., olive pomace) performing

research studies both in lab-scale and in processing mill lines. Barros et al. [15] applied FT-NIR spectrometry (1000–2500 nm) in combination with partial least squares regression for direct, reagent-free determination of fat and moisture content in milled olives and olive pomace; while Allouche et al. [16] used an optical NIR sensor coupled with artificial neural network for online characterization of oil and virgin olive oil to optimize the process. Finally, Giovenzana et al. [17] verified whether vis/NIR spectroscopy could be used to predict the oil content of intact olives entering the mill and of olive paste, pomace, and paté during the milling process.
Multispectral and hyperspectral systems were applied for monitoring the ripening process [18, 19] or on olive oil samples to estimate acidity, moisture, and peroxides by using online system [20] or to discriminate flavored olive oil [21].

4. Portable prototypes and future perspectives toward simplified systems

Having demonstrated the effectiveness of nondestructive analyzes, some problematics remain related to the costs and the dimensions of the instrumentation, two factors that prevent or severely limit some applications of these tools. Research and innovations are allowing these devices to reduce size and weight: devices tend to be more compact and portable. In order to support small producers, systems that are at the same time simple to use and that have a low cost are desirable, so as to make these technologies usable to all and allow real-time evaluations of qualitative and quantitative parameters [22].
Nowadays, chapter authors are working on designing and developing of a simplified LED device for intact olives quality evaluation. A first version of a fully integrated, LED prototype was built and now results patent pending (Figure 1). The peculiar sensory and nutritional characteristics of olive fruits have led to a sharp boost of the demand for the main derivative products in traditional producing areas and elsewhere in the world. Several destructive, expensive, time-consuming, and not sustainable techniques have been used to assess the degree of olives ripeness. To at least partially replace these types of analyses, in 1975, a Maturity Index (MI) has been proposed by Uceda and Frias. This methodology is based on an inexpensive and easy destructive procedure for a visual determination of the best harvesting time.
The method is based on color changes of olive skin and flesh; the protocol foresees to classify 100 olives into eight groups, from intense green (category 0) to black with 100% purple flesh (category 7). Despite this protocol being largely used, MI is highly dependent to the operator experience and could be affected by human error. Moreover, olives color changes are very different among cultivars and during the ripeness evolution.

The aim of this research was to design, build, and test cost-effective and user-friendly devices able to optically predict the olive oil and moisture content in olive fruits in order to support small-scale growers in planning the optimal harvest date. The prototype device is composed of tuned photodiode arrays, interference filters, LEDs, optics and incorporates MEMS (microelectromechanical systems) sensors for spectral measurement in the visible (vis) and short-wave near-infrared (SW-NIR) region.

Therefore, the vision on the application of this sensor can solve several problems in the field of olive growing. Firstly, it can objectify the evaluation of the quality of the olives in the field (to identify the ideal moment of harvesting) and before the milling process to define the correct price of the olives. Secondly, the logistics inside the mill is not easy to be managed. For instance, a preventive evaluation of the maturation parameters could avoid prolonged stop of olives bins in the receiving areas, which causes the deterioration of the product. Finally, the LED prototype could address to olives classification, in terms of qualitative attributes (Figure 2), which is useful for high-added-value olive oil productions.

This new generation of optical devices could be a starting point to build a new concept of cost-effective sensors. The stand-alone instrument should be able to acquire and predict the most important ripening parameters directly from measurements in field. This approach could allow olive maturation monitoring bringing the laboratory directly into the field without picking the olive and reducing sampling waste.

The integration of simple multivariate models in the microcontroller software would be easy calculate and visualize the real-time values of the predicted parameters directly on the device to support operators decision-making with objective numbers.

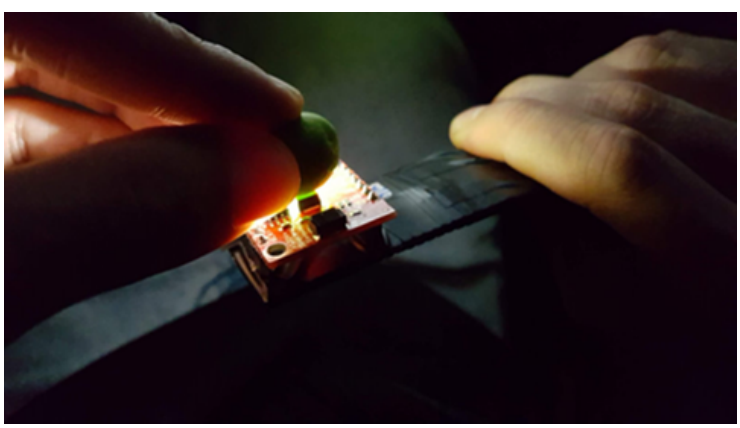

Figure 1. *First version of a simplified LED prototype during optical acquisitions on olives.*

5. Conclusions

Among the different available techniques, vis/NIR and NIR spectroscopy and hyperspectral imaging are valid tools for monitoring of qualitative parameters and for maturation control in olive oil sector. The optical instruments currently on the market are mainly laboratory instruments with dimensions and costs that are not suitable for use in real pre- and post-harvest applications, in particular for SME. To overcome this problem, research has concentrated in recent years on feasibility studies and simulations of simplified systems. These studies have been focused on the preliminary design of systems dedicated to single types of product, aiming at a reduced size and low cost.

At the same time, the development and diffusion of cost-effective and increasingly high-performance hardware have opened up new research opportunities envisaging new systems to support optical measurement for the control and management of the pre- and post-harvest processes. Therefore, further studies both for model improvement and for the design of the system are needed. In a view of olive-growing 4.0, a similar tool based, for example, on a prototype using specific LED for the illumination will lead to quick and accurate analyses in order to get a useful monitoring of the ripening process. In this way it will be possible to estimate the best harvest period and to provide objective features to the operators in terms of quality attributes.

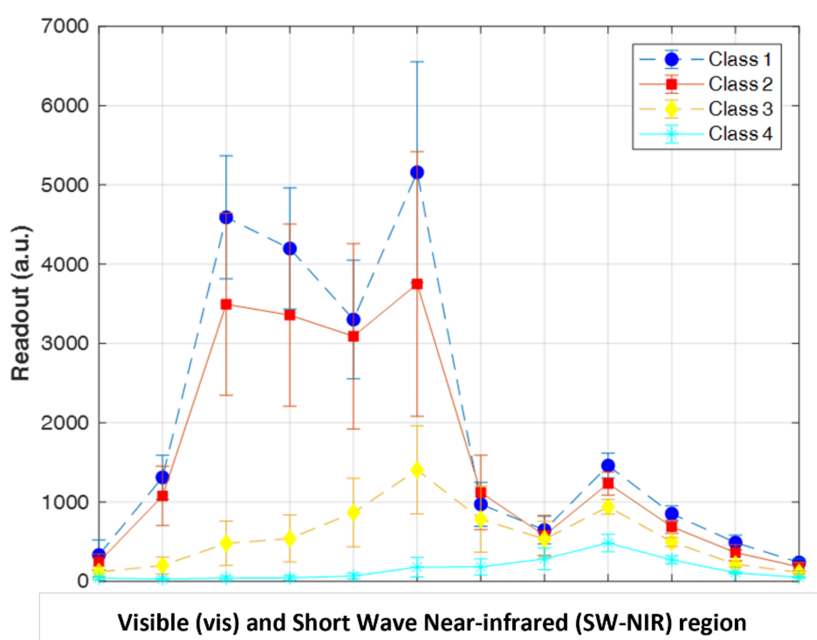

Figure 2. *Average optical readouts and relative standard deviations from each olive ripening class.*

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2.4 USE OF SPECTROSCOPY AND CHEMOMETRICS IN THE AGRI-FOOD SECTOR

Among the various non-destructive techniques mentioned in the previous chapter, this thesis project focuses primarily on visible and Near-Infrared spectroscopy. This technique has proven to be highly versatile, rapid, and potentially applicable in several areas within the agri-food sector. It offers the possibility of obtaining information for quality control using a green method that can be applied at different stages of the supply chain (Cortés et al., 2019). It serves as a valid alternative to complement, and in some cases even replace, the traditional laboratory analyses currently performed.

2.4.1 Vis/NIR spectroscopy basic principles

Spectroscopy has undergone decades of development and has consistently been one of the most crucial physicochemical investigative methods available to the life sciences. It offers high chemical specificity while maintaining a non-destructive nature and can be applied to a broad range of samples (Beć et al., 2022).

Vis/NIR spectroscopy belongs to the domain of molecular vibrational spectroscopy, where the interaction with electromagnetic radiation investigates the vibrational modes of molecules. These modes involve oscillatory changes in bond lengths and bond angles, known as stretching and bending vibrations. Each molecular vibration has specific frequencies that match the wavelengths of the incoming electromagnetic radiation, leading to the absorption of energy and the subsequent appearance of absorption bands in the molecule's spectrum (Beć et al., 2020).

When light interacts with matter, it can cause electronic transitions, vibrational or rotational changes, depending on the energy of the photons involved. These changes occur at specific energy levels, which are characteristic of the particular molecule.

Light, or electromagnetic radiation, consists of waves characterized by the simultaneous propagation of a magnetic field and an electric field, oriented perpendicular to each other. Light possesses both wave-like and particle-like properties, but it is often treated primarily as a wave. The propagation of these waves can be described by several parameters:

- wavelength (λ), which represents the distance between two successive maximums or minimums of the wave, measured in nm;
- speed (v), referring to the rate at which the wavefront advances;
- frequency (ν), representing the number of oscillations that occur at a specific point within a unit of time, measured in Hertz ($Hz = s^{-1}$).

The amount of light absorbed depends on the material's composition. Any object with a temperature above 0°C is capable of emitting infrared radiation. When the radiation interacts with matter, it can be absorbed, leading to vibrations in the chemical bonds within the material. Therefore, the presence of chemical bonds is a prerequisite for infrared absorbance to occur (Smith, 2011). Since the energy carried by a photon is directly proportional to the frequency of the electromagnetic wave, the Planck-Einstein equation can be used to determine the quantity of energy the photon transfers to the material:

$$E = h\nu = \frac{hc}{\lambda}$$

Where:

- E: energy (Joule)
- h: Planck constant (6,63 x 10-34 Js)
- ν: radiation frequency (Hz)
- c: light speed (299 792 458 m/s)
- λ: wavelength (nm)

Wavelength and frequency are inversely proportional: as the wavelength decreases, the frequency of the radiation increases, and consequently, so does the energy. The entire electromagnetic spectrum is divided into distinct regions, each characterized by specific wavelength ranges as shown in Figure 2.4.1.

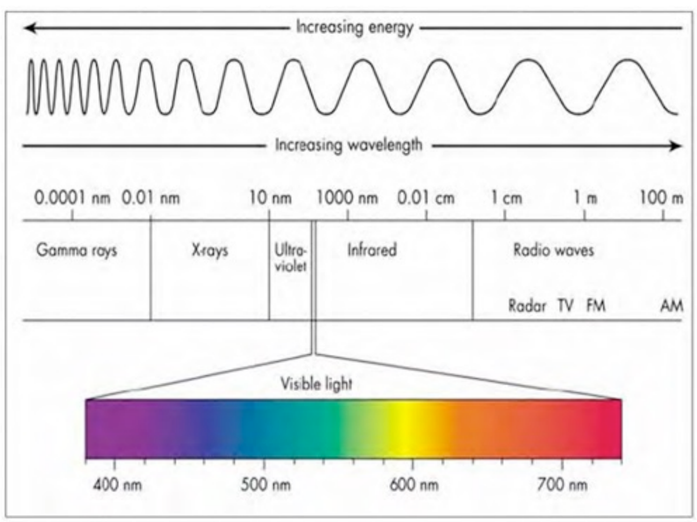


Figure 2.4.1 The electromagnetic spectrum (Lunadei, 2008).

The spectral range between 800 and 2500 nm can be considered as the conventional NIR region, where information about the molecular composition and structure of samples can be obtained.

The vis/NIR region generally corresponds to the portion of the spectrum where the visible region (400-700 nm) and the NIR region (700-2500 nm) overlap (Grabska et al., 2023).
The infrared spectrum is typically divided into:

- Near Infrared (750-2500 nm)
- Mid Infrared (2500-50,000 nm)
- Far Infrared (50-1000 μm)

The Near Infrared region is particularly noteworthy because it targets specific molecular components, facilitating their transition from fundamental vibrational states to excited levels. This process provides valuable insights into the functional groups present in the molecules being analyzed. The absorption of photons by molecules indicates that the energy has been absorbed, and that it is moving towards a lower level of vibrational energy that can be observed through overtones and combination bands in the NIR region.
By applying the Lambert-Beer law, it is possible to correlate the concentration of a sample with the amount of light it absorbs (Figure 2.4.2):

$$A = log\left(\frac{I_0}{I}\right) = log\left(\frac{1}{T}\right) = \varepsilon * l * c$$

Where:

- A: absorbance;
- $\frac{I_0}{I}$: intensity of the radiation before it interacts with the sample;
- I: intensity of the radiation after interacting with the sample;
- T: transmittance;
- ε: specific molar extinction coefficient, which is unique to each molecule;
- l: optical path length of the radiation;
- c: concentration of the sample.

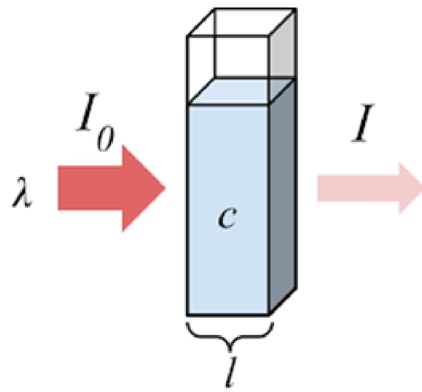


Figure 2.4.2 Lambert-Beer's law.

Typically, the spectrum is represented in a graph with the wavelength on the x-axis and the measurement of radiation intensity on the y-axis, which can be expressed in absorbance, transmittance, or in arbitrary units (a.u.) depending on the instrument used. Absorbance is defined as the amount of light absorbed by a substance and is the negative logarithm (base 10) of the transmittance. Transmittance, on the other hand, is defined as the ability of a material to allow a portion of the incident light to pass through.

2.4.2 Spectroscopy data analysis

One of the fundamental characteristics of using spectroscopy is the intrinsic complexity of the resulting data, a concept that applies to all fields of application, not just the agri-food sector. In addition to being complex, these data often consist of a large amount of information that requires analysis for proper interpretation (Cortés et al., 2019). Therefore, the use of multivariate statistical approaches also known as chemometrics is crucial for extracting information from the spectra since these approaches are really useful when dealing with a large amount of data (Biancolillo et al., 2020). Chemometrics combines mathematical and statistical methods to extract relevant information, distinguishing what is significant from what is data noise (Pasquini, 2003; Beghi et al., 2017).

Data analysis is an iterative process that generally follows the same sequence. First, data are organized into matrices, after being checked to avoid gross errors. Then, data can be mathematically pretreated to reduce the noise associated and minimize artefacts. Artefacts may arise from the measurement modality, instrumental drifts, or other external physicochemical factors (Einax, 2010). Thanks to extensive chemometric research in recent years, several preprocessing techniques are now available to help correct the presence of certain artefacts.

Some pretreatments can be combined, while others may be effective even when used individually (Engel et al., 2013). The user often needs to explore the available options to determine the most suitable technique for the data (Mishra et al., 2020). To solve these problems, several studies have been conducted on the best combinations of pretreatments to use. It is important to consider that using these techniques may also risk eliminating relevant information. While enhancing certain aspects of the spectra, they may compromise the data in other aspects (Xu et al., 2008).
There are several preprocessing techniques suitable for Vis/NIR data (Verboven et al., 2012). Smoothing methods such as Savitzky-Golay smoothing or moving average are used to reduce spectral noise. Standard Normal Variate (SNV) transformation or Multiplicative Scatter Correction (MSC) are applied to minimize another common artefact that can cause vertical baseline shifts, namely additive and multiplicative effects. Methods utilizing the first and second derivatives are employed to enhance resolution and minimize spectral offset and drifts. Scaling and normalization techniques, known also as column pretreatments, such as Mean Centering or Autoscaling, are used to homogenize the data and ensure the correct execution of subsequent data exploration and modeling phases.

Principal Component Analysis

Data exploration is based on methods capable of reducing the dimensionality of the original data, thereby facilitating visualization and enabling the identification of differences between samples (Biancolillo et al., 2020). These are unsupervised methods such as Principal Component Analysis (PCA) or Clustering techniques. Unsupervised methods, in addition to the aforementioned dimensionality reduction, aim to identify structures within the data that would be impossible or extremely difficult for humans to detect. Another goal is to identify errors in the data, such as outliers, that can be removed if necessary.
PCA is a technique that involves transforming the original variables into new variables referred to as principal components. These new variables are obtained through a linear combination of the original variables and each principal component explains a portion of the total variance of the data (Gewers et al., 2021). PCA allows for the evaluation of the actual relevance of the variables while simultaneously reducing the dimensionality of the data by representing them in a new orthogonal space, eliminating spurious information such as instrumental noise, and identifying outliers and/or clusters.
Geometrically, this method involves a rotation of the original data matrix so that the first new axis is aligned with the direction of maximum variance in the data (PC1). The second axis (represented by PC2) is orthogonal to the first and corresponds to the next highest variance direction, with

subsequent axes following in this manner until the number of principal components equals the number of original variables. Although technically the number of principal components can match the original variables, in practice, only the first components capture meaningful information. This is due to variable co-variation with additional components reflecting random noise. As the principal components are ordered according to descending variance, the proportion of explained variance diminishes with each successive component.

PCA allows for the representation of the original data matrix as the product of two matrices: scores and loadings. The loadings illustrate the contribution of each variable in describing the PCs and help identify correlations. In the loading plot the values usually range from 0 (the origin) to 1/-1 (the ends of the axes). Elements near the origin could be not informative, while variables farther from the origin have greater variability and thus generally have more significance. The scores plot, on the other hand, is derived by projecting the original variables with the loadings. The score values represent the new coordinates of the objects reflecting the behavior of the original objects (samples) within the space defined by the PCs (Todeschini, 1998).

<u>Modelling techniques</u>

As for supervised techniques, we can distinguish between modeling approaches that provide qualitative responses, such as classification or class-modelling, and those that offer quantitative responses, such as regression. The primary goal of supervised methods is the prediction of parameters by establishing a relationship, represented by a mathematical model, between the input data (objects) and the target attribute. Classification techniques focus on qualitative discrete variables (categorical), whereas regression techniques address quantitative continuous variables (numerical). When exploring the relationship between a set of response variables measured for objects (Y or dependent variables) and a set of predictors that describe objects (X or independent variables), we refer to this as regression (Biancolillo & Marini, 2018).

The development of a model, or calibration, primarily involves two phases: the development of the model and the application of the model to predict the response in new samples. The mathematical relationship that allows for the prediction of one or more response variables (Y) can be represented by the following formula:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots \beta_n x_n + \varepsilon$$

Where:

- Y = response
- x_1, x_2, x_n = experimentally acquired values of the independent variables, instrumental responses

- $\beta_0, \beta_1, \beta_n$ = regression coefficient values of the model
- ε = experimental error in determining Y

To develop the model, it is necessary to select the samples in the calibration set to cover the entire experimental domain of the predictors. This means that the samples should, as much as possible, represent the sources of variability within the population of samples to be analyzed. The experimentally acquired response values and the reference responses for the samples in the calibration set are used to estimate the regression coefficients of the model.

Various regression methods exist, such as Multiple Linear Regression (MLR), Principal Component Regression (PCR), Partial Least Squares regression (PLS), or Artificial Neural Networks (ANN), which are widely used to predict the characteristics of a sample (Liakos et al., 2018).

The PLS regression method is now one of the most popular linear methods used in chemometrics (Xu et al., 2001). Y has generally been modeled using the MLR method, which is effective when the X-variables are relatively few and not highly correlated. However, with modern measuring instruments like spectrometers, the X-variables often become numerous and highly correlated. When dealing with numerous collinear X-variables, along with response profiles Y, PLS regression allows for addressing more complex questions than before and for analyzing the available data in a more realistic manner (Wold et al., 2001).

The PLS algorithm is similar to the one used for calculating principal components and it aims to identify the latent variables that maximize the covariance between the X and Y spaces. To verify that a model works, it is necessary to validate it and evaluate the fraction of variance it can explain. It is important to consider that increasing a model's complexity also enhances its descriptive capacity (for example, by considering a large number of latent variables), which consequently improves its predictive ability. However, after an initial increase, the predictive ability reaches a maximum and then begins to decline, potentially leading to the phenomenon of overfitting. While adding variables can improve the model's fit to the data, an excessive number can cause the model to focus more on peculiarities related to random fluctuations in the data used for estimation rather than on truly relevant features. Emphasizing a particular group of data might lead to the amplification of background noise and a deterioration of the model's overall predictive power.

Model evaluation

To evaluate the performance of a model, various methods and indices are available. In order to assess the accuracy of the calibration model and to avoid overfitting, validation procedures must

be applied. The calibration model is constructed using the calibration set and the prediction residuals are calculated applying the calibration model to the validation set. Model validation can be performed through internal validation, also known as cross-validation (CV), or through external validation, which involves using an independent external dataset. In external validation, the validation set is independent and can be obtained for example from a sampling carried out with the same characteristics but at a later time (Esbensen & Geladi, 2010).
In the case of internal validation, the dataset is typically divided into two parts: a training set (or calibration set) and a test set. There are different methods to perform cross-validation and to select an appropriate number of cancellation groups for dividing the data into training and test sets. Cross-validation is frequently used as an intermediate step between calibration and external validation with the aim of optimizing model complexity. Alternatively, it can be employed when an external dataset is not available. Cross-validation has been used primarily in chemometrics to estimate the number of components to include in latent variable models (PLS and PCR) for prediction or to estimate model complexity (Esbensen & Geladi, 2010).
There are various mathematical indices available for evaluating the performance of a predictive model. To properly assess a model, these indices must be calculated not only during the calibration phase but also during the validation phase, allowing the selection of the optimal number of latent variables to maximize model reliability, balancing good predictions and overfitting.
According to Nicolaï et al. (2007), model accuracy is usually evaluated using the Root Mean Square Error (RMSE), as well as bias and R^2 (coefficient of determination). A high-performance model should have the lowest possible RMSE and bias, while R^2 should be as high as possible (with a maximum of 1). The RPD index (ratio between the standard deviation of the response variable and RMSE) can also be calculated, providing an immediate indication of prediction accuracy. An RPD value between 1.5 and 2 means that the model can distinguish between low and high values of the response variable; a value between 2 and 2.5 indicates that coarse quantitative predictions are possible, and a value between 2.5 and 3 or higher corresponds to good and excellent prediction accuracy, respectively.

2.4.3 Features and categories of commercial vis/NIR devices

As mentioned in Section 2.3, there are various types of spectrophotometers that can be classified based on the characteristics of some of their components. Another common distinction made when discussing these instruments pertains to their intended use and the environment or situation in which they may be employed. On the market, is possible to distinguish between:

- benchtop instruments, used in research laboratories or industrial laboratories;

- process instruments, used online or inline directly on the production line;
- portable instruments.

Portable instruments, which are a primary focus of this thesis, can themselves be categorized into handheld and stand-alone instruments. However, not all portable instruments are suitable for direct field use. Some are considered portable because they are easy to transport or do not require a direct power source, yet they are not necessarily user-friendly tools that can be operated directly in the field.

Stand-alone instruments, on the other hand, can be placed in the field or at a specific point in a production chain, can transmit data continuously, and can be remotely controlled. Currently, benchtop and process instruments are considered the most reliable, offering the highest resolution; however, they are bulky, very expensive, and often difficult to operate. As a result, there is a growing trend to develop smaller, lighter, easy-to-use, and cost-effective instruments. Portable instruments typically exhibit lower performance compared to benchtop instruments in terms of resolution, range, and signal-to-noise ratio (Crocombe, 2018).

2.5 LCA AND CURRENT METHODS TO ASSESS SUSTAINABILITY IN THE AGRI-FOOD SECTOR

Sustainability stands as one of the paramount challenges of the twenty-first century, encompassing a multitude of realities and sectors. Notably, the food industry occupies a pivotal position, both due to the intensive exploitation of land and resource consumption, as well as the significant environmental and social impacts associated with production and supply chain activities.

In this context, the concept of a circular economy (CE) is introduced, which involves creating circular loops to maximize the value of materials and energy within production and consumption systems (Moraga et al., 2019). The goal of a circular economy should be to establish virtuous loops that recycle and reuse materials instead of consuming virgin resources (De Pascale et al., 2021). Achieving these objectives requires the regeneration of natural systems and the proper organization of waste flows. The implementation of sustainability and CE models in the agri-food sector should be guided by action plans supported by specific indicators, serving as essential tools for assessing the developments of enterprises and systems (Silvestri et al., 2022).

According to De Schoenmakere & Gillabel (2017), for the sustainable management of the agri-food system, it is crucial that the indicators and evaluation tools employed consider the entire life cycle of a product, as well as the social context of reference. A life-cycle approach is necessary to conduct a comprehensive and accurate analysis of the entire system or supply chain.

Life Cycle Assessment (LCA) is a crucial tool for analyzing the environmental sustainability of products and technologies, providing a systematic approach to improving resource efficiency and promoting cleaner production. Defined by ISO standards as a method to assess a product's environmental impact from "cradle to grave," LCA is particularly effective in the agri-food sector, allowing for the evaluation of environmental effects across the entire value chain, from production to consumption. LCA is widely used to assess the environmental impact of agricultural systems, food processing, and production activities, often compared to alternatives such as food waste management. However, achieving comprehensive sustainability in line with Circular Economy (CE) principles also requires integrating other Life Cycle Thinking (LCT) tools, like Life Cycle Costing (LCC) for economic impact assessment and Social-LCA for evaluating social impacts.

The United Nations Sustainable Development Goals (SDGs) (Nations, 2015) have underscored the importance of methodologies that capture both the negative and positive social impacts of value chains. Despite this, there is still limited research on the application of S-LCA and LCC in the agri-food sector, with only a small percentage of studies addressing these approaches.

The agri-food sector faces challenges in selecting appropriate sustainability indicators due to the variety and complexity of potential measures. While there is extensive literature on sustainability and CE indicators, there is a lack of a comprehensive framework specifically for the agri-food sector, highlighting the need for further research and development in this area.

Indicators to evaluate sustainability in the agri-food sector:

To implement corrective actions or modifications in the production chain of a food product to enhance its sustainability, it is essential to measure and evaluate sustainability as objectively as possible. Various indicators and calculation methods exist that can address the three main dimensions of sustainability: environmental, economic, and social. These indicators can be employed to measure and monitor the sustainable performance of companies or agricultural practices throughout the entire production chain. They can vary based on the context and specific objectives and can be combined to create a comprehensive overview of a supply chain. Generally, they include:

- Carbon Footprint, focuses specifically on quantifying the greenhouse gas emissions associated with a product or service, it is often a component of a broader LCA study but can also be conducted independently;
- Water Footprint, quantifies the total volume of water used (consumed and polluted) during production, including the water needed to grow raw materials, it is highly relevant in this sector, particularly in agriculture, where water use is a critical sustainability issue;
- Land Use, assesses the amount of land required for agricultural production, with particular attention to deforestation and soil degradation;
- Biodiversity, measures the impact of agricultural practices on the diversity of plant and animal species, contributing to the conservation of ecosystems;
- Energy Efficiency, calculates the energy used to produce a certain quantity of product, aiming to minimize energy consumption to improve sustainability;
- Resource Efficiency, measures the efficiency in the use of natural resources, such as energy, water and fertilizers in food production;
- Production Cost: Analyzes the economic costs of production, including the costs of resources and materials, to ensure economic sustainability;
- Working Conditions and Social Well-being, evaluate the impact of agricultural practices on working conditions, safety, and worker well-being;
- Impact on Local Communities, measures how production activities affect local communities in terms of economy, health, and quality of life.

Environmental Product Declarations (EPDs) can also be included, which are standardized documents that communicate the environmental impacts of products based on LCA. EPDs are essentially a calculation method that includes several indicators and are becoming more common in the food sector to provide transparency to consumers and stakeholders. Sustainable Agriculture Certifications, such as Organic, Fair Trade, and Rainforest Alliance, often incorporate LCA principles or elements of sustainability assessments to ensure that products meet certain environmental and social criteria.

LCA and these associated methods are integral to understanding and improving the sustainability of products and services in the agri-food sector. They provide a robust, science-based approach to assessing environmental impacts and guiding sustainable development.

<u>What is LCA?</u>

Life Cycle Assessment is a methodology to evaluate the environmental sustainability of products, processes, or services throughout their entire life cycle.

This methodology represents the primary operational tool of Life Cycle Thinking. It is an objective method for assessing and quantifying the energy and environmental burdens, as well as the potential impacts associated with a product or service. One of the key aspects that makes this type of study innovative is its evaluation of all phases of a production process as interconnected and interdependent. Internationally, the LCA methodology is regulated by ISO standards 14040 and 14044, according to which a life cycle assessment study involves: the definition of the goal and scope of the analysis; the compilation of an inventory of inputs and outputs for a given system; the evaluation of the potential environmental impact associated with these inputs and outputs; and the interpretation of the results.

According to ISO 14040 and 14044, an LCA study consists of four interconnected stages:

1. Definition of the goal and scope
2. Inventory analysis
3. Impact assessment
4. Interpretation of results

The diagram below graphically represents these four phases:

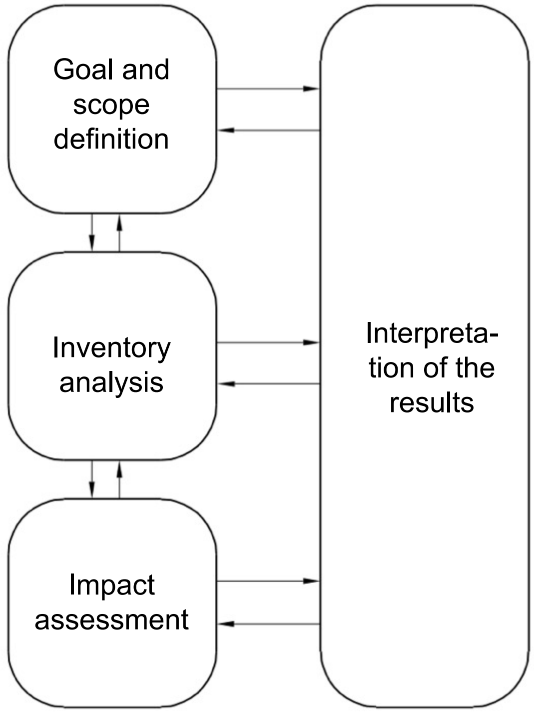


Figure 2.5.1 Four phases of a Life Cycle Assessment study.

1. Goal and scope definition

The purpose of this phase is to define the objective of the study, the products under analysis, the system boundaries, the functional unit, and all relevant assumptions. The system boundaries are often represented by a general diagram showing the flow of inputs and outputs, including all operations contributing to the life cycle of a product, as well as the processes and activities that fall within these system boundaries. The purpose of the functional unit is to provide a reference unit against which all inventory data can be normalized. The definition of the functional unit depends on the category of environmental impact and the objectives of the analysis; it is typically related to the mass of the product being examined.

2. Inventory analysis

This phase involves data collection. All data concerning transportation, raw material extraction, processing, and the production of materials used are gathered. These types of data can also be applied to productions not specifically related to the product in question, such as data on electricity production, coal, and packaging. A distinction is made between:

- primary data, specific to the analyzed product, directly collected in the field;

- secondary data: derived from other sources, such as professional databases or scientific articles.

3. Impact assessment

The goal of the impact assessment (LCIA) is to understand and evaluate the environmental impacts based on the inventory analysis, within the framework of the study's goal and scope. In this phase, the inventory results are assigned to specific impact categories.
Impact assessment in an LCA study generally follows several steps (ISO 14040, 2021):
- election of impact categories
- classification
- characterization
- normalization
- evaluation.

4. Interpretation of the results

The objective of an LCA study is to arrive at conclusions that ensure a reliable outcome, fulfilling the study's goal and scope (ISO 14040:2021). The final phase involves identifying, quantifying, controlling, and evaluating the information obtained from the inventory analysis and impact assessment, to communicate it effectively. This final evaluation might also include qualitative and quantitative improvement measures to reduce the environmental impact of the system under consideration, such as raw material use, industrial production, consumer use, and waste management.

Key aspects of LCA in the agri-food sector:

In the agri-food sector, LCA is employed to assess the environmental impacts from the production of agricultural inputs to the consumption and disposal of food products. This approach considers multiple stages, including cultivation, processing, packaging, transportation, and waste management, providing a comprehensive picture of environmental burdens such as greenhouse gas emissions, water and energy use, and pollution. LCA's structured framework allows for the identification of hotspots in the supply chain where improvements can yield significant sustainability benefits. It considers a variety of impact categories, including global warming potential, eutrophication, acidification, and resource depletion. By quantifying these impacts, stakeholders can make informed decisions to enhance sustainability practices and policies.

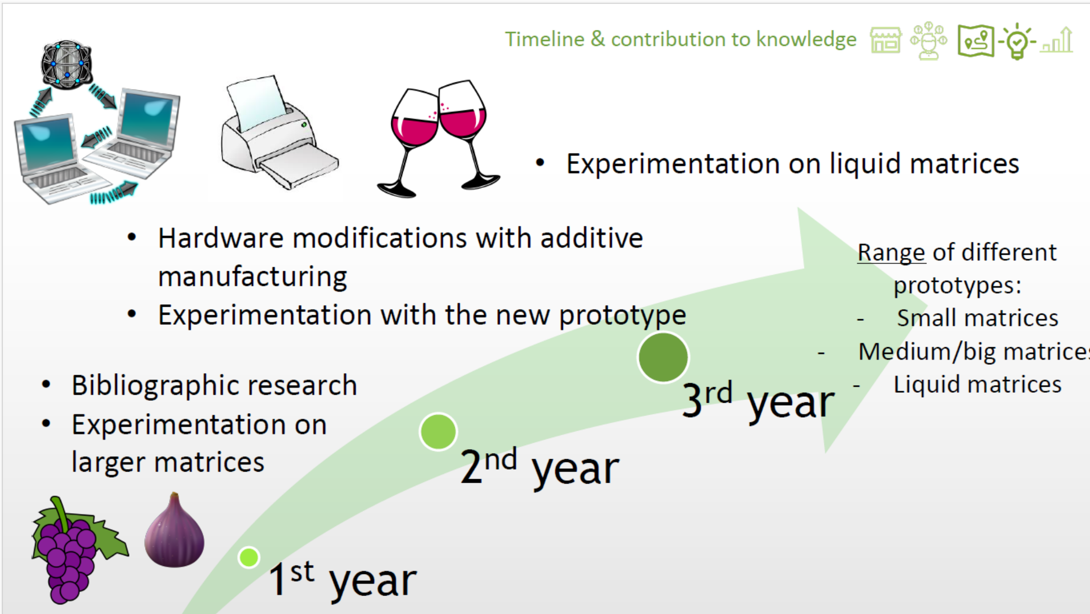
3. AIMS and OBJECTIVES

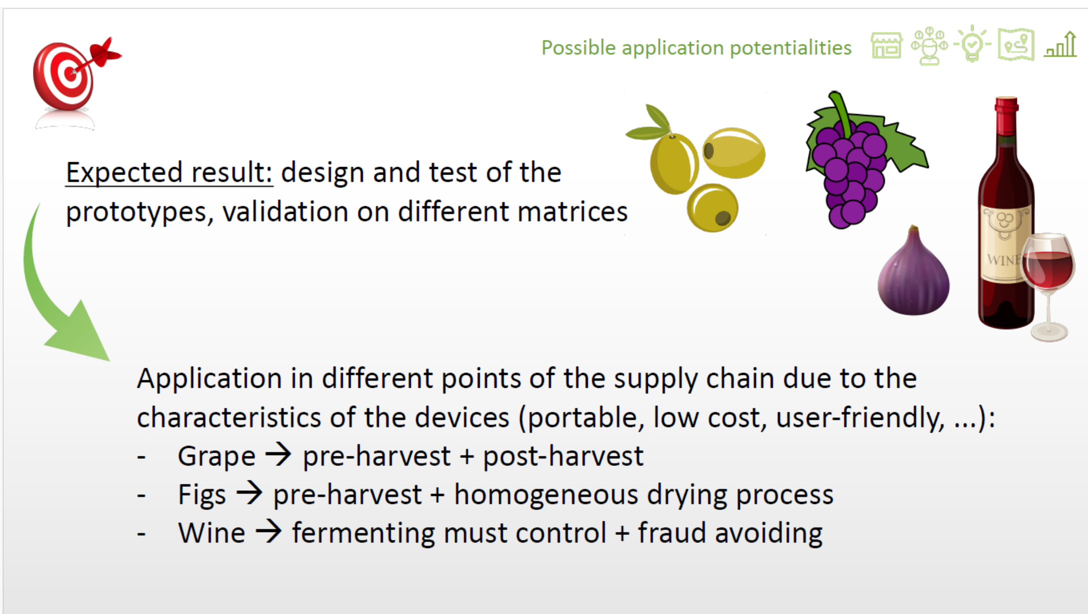
The goal of this PhD research project was to propose innovative solutions for objectively measuring quality attributes of different agri-food products using simplified portable devices that apply visible/Near-Infrared (vis/NIR) spectroscopy. This technique, already widely used in the agri-food sector, typically requires complex and expensive instrumentation. The development of new smart sensors could improve production monitoring, increase awareness among supply chain operators, enhance resource management, and reduce waste. Waste reduction, in particular, is a crucial goal for the entire agri-food supply chain. Moreover, it is essential for achieving Goal 12 of the 2050 Agenda in terms of substantially reducing waste production.

At the beginning of this PhD journey, the activities were organized as follows: after in-depth bibliographic research and starting from experiments already in progress on small matrices (initially olives and subsequently grapes), the aim was to move on to experiments on larger matrices and to identify the necessary modifications to the first version of the prototype. The intention was then to create a prototype for liquid matrices as well, so that by the end of the PhD, a range of three prototypes with different characteristics would be available.

During the experimentations, not only portable instruments were used. Several experimentations involved the use of benchtop instruments that served as reference for the optical acquisitions obtained using the prototypes, as well as for conducting feasibility studies and with the aim of implementing this type of instrumentation in-line within the food industry.

Timeline and expected results declared during the first Journal Club (23 February 2022):





4. RESULTS CHAPTERS

4.1 DEVELOPMENT OF A PORTABLE OPTICAL PROTOTYPE

In this first chapter of the results, two published research articles are presented.

The first experimentation, conducted on tomatoes, was carried out using the prototype in its pre-prototype stage. Based on this research work and other studies conducted on olives and different matrices (Tugnolo et al., 2021; Pampuri et al., 2021), structural modifications were made to the first version of the device.

Research activities have shifted to the viticulture and oenology sector because there is an increasing presence and use of this type of sensor in this field, where they can achieve significant success.

In this sector, it is crucial to monitor the maturation of grapes to produce a high-quality final product. Until now, the monitoring of maturation has mainly relied on wet chemical analyses which, unlike optical techniques, are destructive, time-consuming, require specialized personnel, and involve complex sampling processes. Some optical instruments have been used in viticulture laboratories for several years, but research is now focusing on portable instruments that allow analyses to be transferred from the laboratory to the field. Specifically, for analyses of technological and phenolic maturation, it has been demonstrated that proximal or remote optical sensors can enable the assessment of vine conditions directly in the field (Power et al., 2019).

The optical prototype in his final clump version, as shown in the second research paper, was patented for the analysis of small food matrices (patent application PCT n. PCT/IB2022/051110 for Portable Device for Analysing Vegetable Matrices on the Field and Related System and Method).

PAPER 1: Test of a light emitting diode fully integrated pre-prototype spectrometer for rapid evaluation of table tomato (*Solanum lycopersicum* L., Marinda F1) quality

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Abstract
The present research aims to evaluate the performance of an optical pre-prototype based on light emitting diode, (450-860 nm) to quantify table tomatoes' quality features in a rapid and non-destructive way (Solanum lycopersicum L., Marinda F1). A total of 200 samples were analysed. Calibration of the pure near infrared (NIR, 960–1650 nm) and visible/near infrared (VIS/NIR, 400–1000 nm) commercial spectrometers to estimate the main tomato quality parameters, i.e. moisture content (MC) and total soluble solids (TSS), was performed by using PLS regression. Since no substantial differences were highlighted between the two commercial devices, to reduce the complexity while keeping the performance of the model using the whole spectra (1647 variables for VIS/NIR), a cost-effective pre-prototype was designed and built by using 12 bands in the VIS/NIR optical range. The pre-prototype shows slightly lower performance, resulting in RMSEP values of 2% and 1.45 °Brix for MC and TSS respectively, compared to RMSEP values of 1% and 1.19 °Brix for the VIS/NIR device (using the entire spectrum). Moreover, no significant differences at 95% were highlighted by using Passing-Bablok regression. In conclusion, the pre-prototype performance can be considered sufficiently accurate to allow an initial field screening of the trend of the analysed parameters (MC and TSS) using a new generation of simplified optical sensors.

Keywords
Simplified optical device, ripening, visible/near infrared and near infrared spectroscopy, instrumental comparison, chemometrics

Introduction

Tomato (Solanum lycopersicum) is the second most-produced and consumed vegetable in the world and its global production in 2019 reached almost 197 million tons (1).

Concerning tomato production, in 2016 Italy has been the third larger producer (5.2 million tons of processed tomato) in the world behind California and China. Half of the Italian tomatoes are grown and processed in Northern Italy, in particular in Lombardy, Veneto, Piedmont and Emilia-Romagna (2). Due to the great importance of this cultivation, it is essential to identify innovative solutions that maximize crop production and at the same time reduce waste. Technology-based solutions may be confounded by the large number of species and the complexity of plant-environment interactions within crop production systems. Therefore, the development of new approaches for improving understanding of crop biology to maximize production, minimize losses and to improve pre and post-harvest production and utilization is a crucial aspect (3).

Among the new approaches, it is well known that in recent years research has been focusing on non-destructive spectroscopic methods capable of exploring many samples and providing a complete and rapid overview of the maturation of fruit and vegetable products. Furthermore, spectroscopy, combined with multivariate management of the data, is a powerful analytical method that doesn't require any treatment to the sample and can be integrated on existing machinery for an increasingly automated quality control. (4). However, most of the studies reported on tomatoes involve the use of expensive benchtop instruments that cover the entire spectral range of visible and near infrared (between 400 nm and 2500 nm approximately). Some recent studies have reported the possibility of using NIR spectroscopy for the determination of total soluble solids (TSS), titratable acidity and carotenoid compounds in salad tomatoes (5–7), while hyperspectral imaging has been applied for the estimation of moisture content, pH and TSS (8). A portable spectrometer was used by Sheng et al. (2019) (9) to predict soluble solids and lycopene in cherry tomatoes at different temperatures and by Arruda de Brito et al. (2021) (10) to develop models for intact tomatoes' TSS.

Over the last three decades, researchers have looked into the possibility of developing simplified optical devices for specific applications (11, 12), and focusing on the latest years the research has been moving towards portable, inexpensive, easy and quick to use instruments that do not need the entire spectral range to provide useful information (13). Internet of things, big data and artificial intelligence and their disruptive role in shaping the future of agri-food systems (e.g. greenhouse monitoring, intelligent farm machines, drone-based crop imaging, food quality assessment using spectral methods), are making an impact only in very recent times, thanks to the advent of industry 4.0. In the IoT framework, proximal-remote sensors gather information generated by machines to increase efficiency, promote better decision-making and build competitive advantages, regardless of industry or company size (14).

Miniaturized sensors enabled a new and previously unattainable spectrum of applications of NIR spectroscopy, agriculture and the food sector, materials science, industry and environmental studies, having an impact on operational characteristics, marking a significant turning point in the evolution of the practical applications of NIR spectroscopy (15). In contrast to a mature benchtop spectrometer sector, the handheld devices are much less uniform and incorporate various novel technologies resulting in different performance, with narrower spectral regions, lower resolution, leading applicability limits and lower analytical performance (16).

In the agri-food sector there is still a need to develop customized cost-effective solutions for specific applications and the set of characteristics required must be merged together in devices and applications that are not actually already available. Also considering complementary but fundamental aspects as (i) the development of specific multivariate calibrations already on board the devices, and (ii) the optimization of the interconnection of the devices, e.g. cloud data storage and cloud computing. The maturity of the tomato, in particular for small companies, is normally evaluated basing on the experience and on the color of the surface perceived by the human eye, in this way the fruits could undergo overripe. On the contrary, if the tomatoes are picked too early, they will not reach the desired ripeness, also causing an economic and technological (in the case of tomatoes intended for processing) damage. The case of tomatoes is even more particular than other fruits because there are varieties that reach maturity in the absence of the color change towards red: detection of mature-green and immature-green tomatoes has been a challenge for researchers since there is no difference in terms of external appearance of the fruit (17).

The accurate and objective judgment of the maturity and harvest time is a critical prerequisite to maintain the quality of tomatoes. Additionally, supply chain operators may also need tomatoes with different maturities to meet various commercial purposes, so the harvest standard of tomatoes is various. However, in terms of the producers, it may not be able to accurately determine the optimum harvest time due to the large subjective error. The quality grading for the picked tomatoes through the use of innovative optical sensors could be an effective method to improve the agricultural output value and reduce the economic loss (18).

Therefore, the aim of this work was to test a miniaturized LED fully integrated pre-prototype for rapid evaluation of MC and TSS of table tomatoes (Solanum lycopersicum L., Marinda F1) in a rapid and non-destructive way. Moreover, a statistical comparison with a commercial portable VIS/NIR device was carried out to verify the effectiveness and reliability of this new generation of simplified optical sensors.

Materials and methods

Sampling

The experimentation was performed on table tomatoes Solanum lycopersicum L., Marinda F1, a variety easily available on the market at different stages of maturation. In order to represent the ripening process, in May 2019, a total of 200 tomato samples were bought and analyzed over 3 weeks. Three arbitrary ripening classes (figure 1) were created according with the skin color: totally green (extremely unripe, class t1, 50 samples), green/yellow/orange/red surface (medium ripe, class t2, 100 samples due to the tomatoes variability), totally red surface (ripe, class t3, 50 samples). Samples were processed in a laboratory within a few hours of purchase to acquire optical spectra and to perform moisture content MC (%) and TSS (°Brix) used as wet-chem reference parameters.

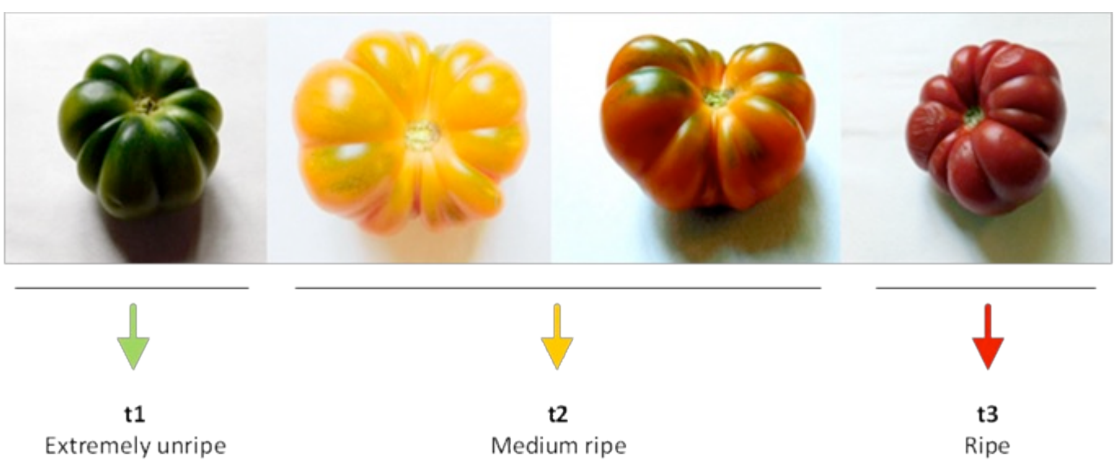

Figure 1. Ripening tomatoes classes (from left, class t1, class t2, and class t3).

Optical analysis

Optical analyses were performed (before the wet-chem analyses) on tomato without any sample preparation. Each tomato sample was analyzed using two commercial portable spectrophotometers: NIR (Aurora NIR, Grainit, Italy) and a portable VIS/NIR (Jaz Modular Optical Sensing Suite, OceanOptics, Inc., Dunedin, FL, USA). The NIR spectrophotometer is equipped with a halogen light source, an InGaAs sensor in the NIR module (960 - 1650 nm, spectral resolution 10 nm) and it is designed for diffuse reflectance acquisition with automatic internal calibration. The VIS/NIR spectrophotometer (400 - 1000 nm) works using a bifurcated fiber that conveys the light from the halogen lamp to the sample and back to the detector. The tip of the fiber was equipped with a cap that standardized the analysis distance (about 2 mm) and reduced the environmental light interference. The background consists of white (100% of light reflected) and black (0% of light reflected) standards. An integration time of 50 ms was set in order to collect the best spectral dynamics using a light intensity of 3000 lumens. Spectral measurements were taken from three points on each sample, apical, central, and basal area, and the values were averaged to obtain a mean optical spectrum for each sample.

Then, based on the results, a LED fully integrated stand-alone pre-prototype (technology readiness level estimated equal to 3, figure 2), designed by the Department of Agriculture and Environmental Sciences of the Università degli Studi di Milano, were tested on tomatoes.

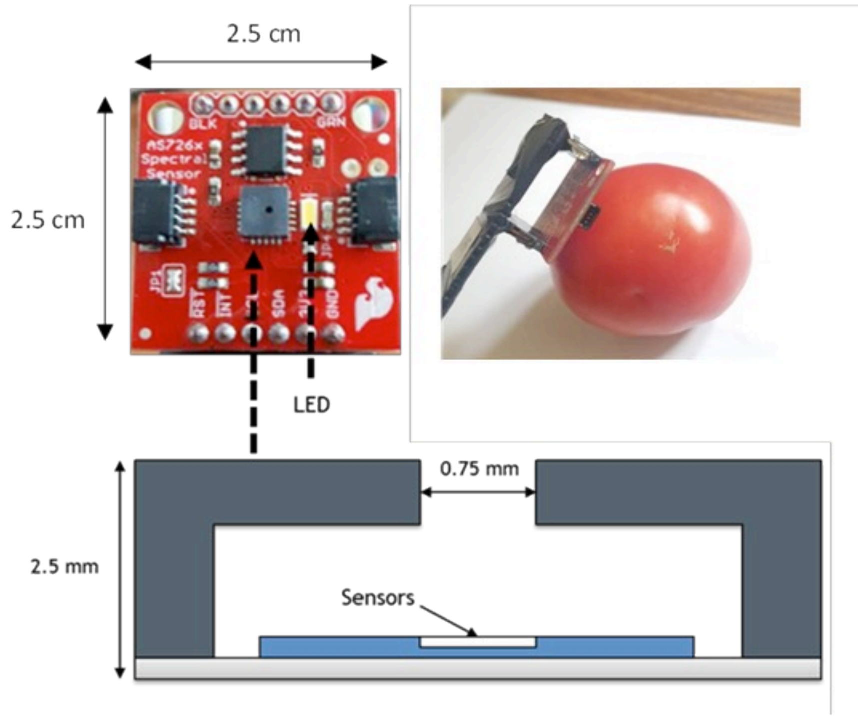

Figure 2. LED fully integrated stand-alone pre-prototype (TRL3) managed by using a portable computer.

The proposed prototype is composed by tuned photodiode arrays, interference filters, LEDs and optics. In detail, the device incorporates two sensitive spectrometers (6 optical channels each one, dimensions indicated in Figure 2) available in the form of breakout boards (AMS, models AS7262 visible and AS7263 NIR, Premstaetten, Austria-Europe), which include sensors and auxiliary electronic components for spectral measurement in the visible (VIS) and Short Wave Near-infrared (SW-NIR) region, have been used (Fletcher & Fisher, 2018). The sensors are 4.5 × 4.4 mm in size and are classified as ultra-low power consumption sensors. They have a 16-bit radiometric resolution and 12 independent on-device optical filters from 450 nm to 860 nm as summarized in table 1. The prototype enables chip-scale spectral analysis by integrating Gaussian filters into standard complementary metal-oxide-semiconductor (CMOS) silicon via nano-optic deposited interference filter technology. The six-channel VIS sensor (VIS module) is sensitive to the 400 - 700 nm spectral range with center wavelengths of 450, 500, 550, 570, 600 and 650 nm (interesting to get also color information, a crucial feature to get indications regarding the ripening progress). While, the six-channel SW-NIR sensor (NIR module) is sensitive to the 600 - 900 nm spectral range with center wavelengths at 610, 680, 730, 760, 810 and 860 nm. Each module is composed of 6 independent optical filters whose spectral bandpass is defined with full-width half-max (FWHM) of 40 nm for the VIS module and 20 nm for NIR module.

The sensors can read the intensity of light at the 12 wavelengths (6 for each module) and give digital output (readout) corresponding to the intensity of light falling on it. The light source is a white super bright LED illumination (5700K) with an irradiance of ~600μW/cm2. The light detection position is in contact with the fruits, while the LED emission position is on the side at about 2 mm from the surface of the tomato.

The pre-prototype has been configurated using Arduino to perform an average of 10 scans for each acquisition point in order to reduce the experimental noise.

Table 1. Sensor wavelengths.

	Wavelengths (nm)					
Sensor 1	450	500	550	570	600	650
Sensor 2	610	680	730	760	810	860

Chemical analyses

After optical measures, the wet-chemical analyses (reference parameters) were also performed on the same samples. Each tomato was centrifugated and the juice analyzed for a representative measure of whole tomato.

The analytical methods to determine the moisture content (MC) recommend a temperature of 103°C for 24 h, until constant weight of product (19). The samples were weighed with a balance (LAZ 30P, Sartorius Lab Holding GmbH, Goettingen, Germany) and dried with a laboratory oven (UNB400, Memmert GmbH & Co, Schwabach, Germany) in order to determine the dry weight of samples collected. Once reached room temperature, samples are re-weighed (dry weight) for the MC determination (Eq. 1):

$$MC(\%) = \frac{\left(m_{gw} - m_{dw}\right) * 100}{m_{gv}}$$

Where:

MC (%) = percentage of moisture content.

m_{gw} = gross weight.

m_{dw} = dry weight.

The TSS content was evaluated by using a digital refractometer (PAL-1 ATAGO, Tokyo, Japan, accuracy refractive index ±0.2 °Brix) measuring the refractive index of the juice (°Brix).

Data processing

The entire data analysis was performed in the Matlab® environment, version 2019b (The MathWorks, Inc., Natick, MA, USA) using PLS-Toolbox package (Eigenvector Research, Inc. Manson, Washington) and Passing and Bablok regression by Andrea Padoan (Jan 16, 2010). The reference parameters (MC and TSS), representative of tomato ripening process, and the optical diffuse reflectance data obtained from NIR and VIS/NIR commercial spectrophotometers were analyzed in a multivariate way to (i) qualitatively understand the relationships among all variables and among variables and samples, i.e. principal component analysis (PCA), and to highlight outliers based on a detection procedure applied on PCA scores using the 'Hotelling T2 computation' function (α value was set to 0.05), (ii) quantitative predict the reference parameters, i.e. partial least square regression (PLS). The logic scheme of data processing is presented in figure 3.

Moreover, in order to reduce the instrumentation complexity keeping the performance of the model built using the whole spectra (1647 variables for VIS/NIR), a cost-effective pre-prototype, characterize from 12 bands (table 1) was tested and the same data processing approach (figure 3) carried out. Afterwards, for a better understanding of the practical applicability of the proposed LED technology for the quality parameters prediction (MC and TSS), the Passing-Bablok regression method was applied on the PLS prediction outcome deriving from VIS/NIR instrumentation and pre-prototype device.

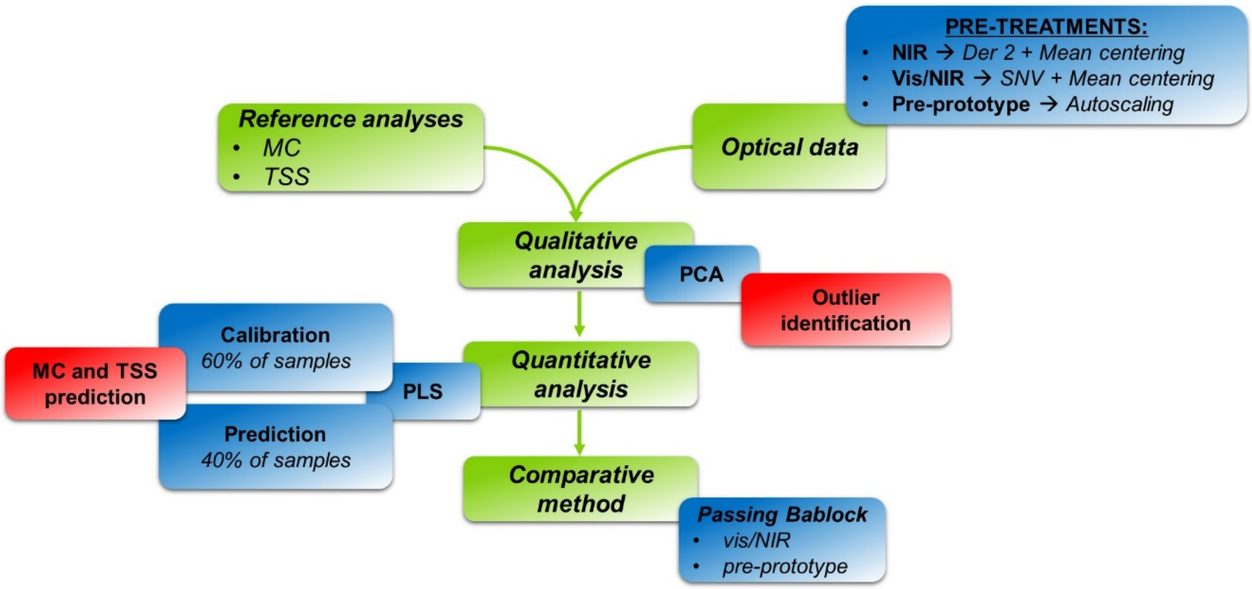

Figure 3. The logic scheme of data processing (green), methods applying (blue) to obtain results (red).

Before applying multivariate analysis, the optical data were pre-treated to reduce instrumental noise and the scattering effects (due to the inhomogeneous physical structure of the tomato's samples, size and shape, and the various positions and distances where the sample can interact with the sensor).

On NIR spectra, second derivative (Der 2) and mean centering were applied. Der 2 performed by using Savitzky-Golay algorithm (filter width = 15, polynomial order = 2) which enhances the separation of overlapping peaks allowing more specific identification of small and nearby lying absorption peaks which are not resolved in the original spectrum, thereby offering means to increase the selectivity of absorption peaks for certain molecules of matrix. Der 2 was applied paying attention to avoid suppression of broad bands and enhancement of noise. Mean centering ensures that all results will be interpretable in terms of variation around the mean. For VIS/NIR optical data a correction of the baseline vertical shifts (offsets) and of the global intensity effects (typically arising from unwanted light scattering) was performed applying the Standard Normal Variate (SNV) transform and mean centering.

The data obtained from the two sensors of pre-prototype were processed and analyzed as data obtained from one single pre-prototype in order to take advantage of all wavelengths considered.

A data scaling phase was applied to the readouts of pre-prototype, in order to make the different variables comparable in importance before applying scale-dependent multivariate analysis methods (such as PCA or PLS). For this purpose, the unit variance scaling (or autoscaling), variables are divided by their respective standard deviations, was applied. The method is commonly used to data sets containing variables with different units and scales in order to impose equal weights in the analysis (20,21).

Then, the reference parameters were used for the calculation of PLS predictive models. Kennard–Stone algorithm (22) was applied to select a representative subset to ensure training samples spread evenly throughout the sample space. In this paper a 200 samples set was partitioned into training (60% for calibration set, 120 samples) and test (40% for prediction set, 80 samples) sets. To identify the most suitable pre-treatments and the number of latent variables (LV), the models were evaluated using an internal validation (Cross-validation) through the Venetian Blinds splitting method with five cancellation groups. To measure the PLS models accuracy, the statistical parameters used were the RMSE (root mean square error, RMSEC, in calibration and RMSEP in prediction), as well as latent variables, bias, and R^2 (determination coefficient).

Methods comparison

In order to compare the practical applicability of the pre-prototype device to predict quality parameters correlated to the tomato ripening, the Passing-Bablok regression (23) method was applied on the MC and TSS values obtained by pre-prototype and the commercial VIS/NIR spectrometer on the same dataset, i.e. the prediction set. Since the Passing-Bablok regression method is a symmetrical non-parametric technique, which can build regression models also when both variables (independent and dependent) have a non-negligible experimental error, the regression method results particularly suitable for method comparison. For statistically evaluating the similarity/diversity between these two independent estimations, slope and intercept of the fitted line were calculated, and a significance test was conducted. The null hypothesis (H0) was verified when the slope was not significantly different from 1 and, simultaneously, the intercept was not significantly different from 0, meaning that there are no significant differences between the two methods, at a 95% confidence level (24). Hence, the Passing-Bablok regression allows to evaluate if the performance deriving from pre-prototype are equally comparable to performance from commercial VIS/NIR instrumentation for the quantification of tomato quality parameters.

Results and discussions

Reference data analysis

The boxplots in figure 4 provide a visualization of summary statistics for MC (figure 4a) and TSS (figure 4b) obtained using the destructive reference analysis on the tomato's samples at each sampling time. The mean, median, the interquartile range, and the data range were represented into the graphs. Moreover, the potential outliers (observations beyond the data range whisker length) were also reported. By default, a potential outlier is a value that is more than 1.5 times the interquartile range away from the bottom or top of the box.

Firstly, no apparent differences were highlighted for MC which ranged about 83% to 93% during the three sampling times (median of 90% at t1, 87% at t2 and 88% at t3).

Instead, concerning TSS, an increasing trend from a median of 6.7 to 10 °Brix was observed along sampling, representing the ripening process. Moreover, a very high variance, strictly related to the higher number of samples (100 samples) and to the different features of the medium ripe samples, was captured for the samples analysed at t2 (from 4.8 to 14.8 °Brix).

Finally, three and two potential outliers were identified in the reference data for MC and TSS, respectively.

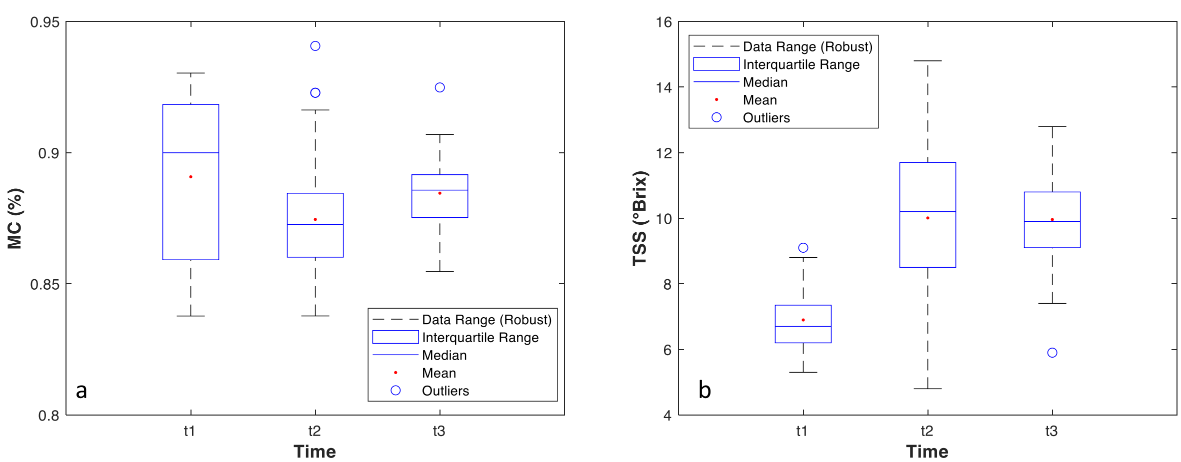

Figure 4. Box-whisker plots of the reference data for (a) moisture content and (b) total soluble solids obtained from the tomato samples.

Spectra exploration and regression

Figure 5 shows raw and pre-treated spectra obtained from the two commercial VIS/NIR (450-950 nm, Figure 5a) and NIR (950-1650 nm, Figure 5c) spectrophotometers. A visual analysis of the spectra highlights the main absorption bands related to the main constituents in tomato's samples. The visible range exhibits clear differences between the different tomato maturity groups around 550 nm and 675 nm, which are mainly related to the variation of anthocyanin and chlorophylls. As the maturity of tomato advances from green to red, its chlorophyll content decreases, while anthocyanin increase (13). Moreover, in the short-wave near-infrared (SWNIR), an absorption band at 760 nm (caused by the third overtone of OH stretching) is noticeable (23). While, in the pure NIR region (from the NIR spectrophotometer, figure 5c), the main absorption bands are related to the stretching of the CO bonds of aliphatic esters, to the second overtone of CH stretching vibrations of alkyl groups and alkenes (1212 and 1245 nm) and the water absorption related to the OH stretch first overtone around 1440 nm (25–27).

However, due to the nature of the samples and to the use of portable devices, a scattering effect was highlighted in the raw spectra. Therefore, two different pre-treatments (SNV for VIS/NIR spectra and second derivative for the NIR spectra) were applied in order to ensure a correction of the global intensity effect – scattering at the sample surface (Figure 5b) and the baseline drift, typically, ascribable to the use of portable devices (Figure 5d).

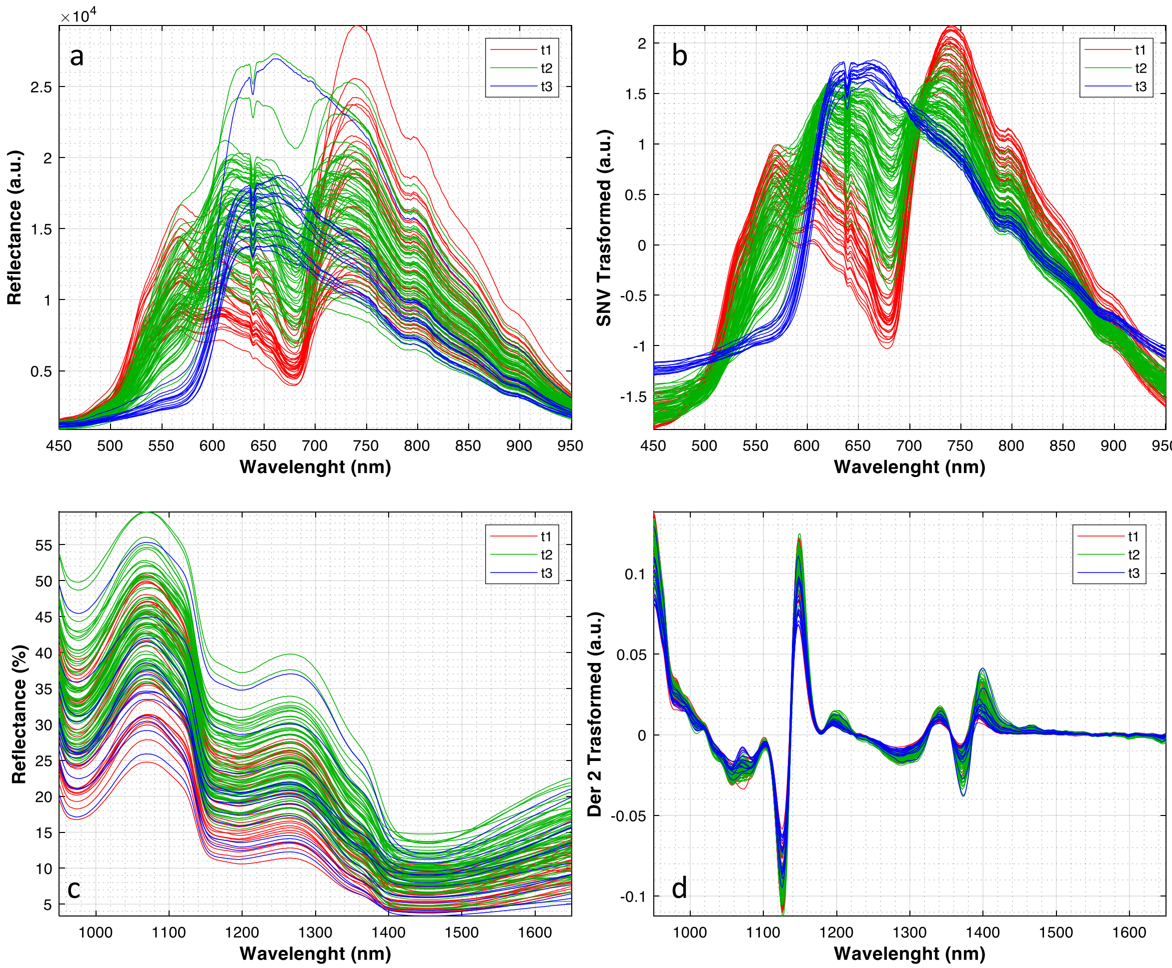

Figure 5. Raw (5a, 5c) and pre-treated (5b, 5d) spectra (standard normal variate for visible/near-infrared spectra and second derivative for near infrared spectra spectra) obtained from intact tomato samples.

PCA was performed on both types of optical data (data not shown) allowing to detect one and two possible outliers in the VIS/NIR and NIR spectra, respectively. Afterwards, regression models (by means of the PLS method) were developed for the prediction of MC and TSS starting from the data coming from the two different type of portable spectrophotometers. The parameters used for evaluating the model goodness are presented in Table 2. Overall, regarding R2 and RMSEC and RMSEP, relative slightly low differences between the performance of the NIR models in calibration (R2Cal = 0.82 and RMSEC = 0.01 for MC and R2Cal = 0.84 and RMSEC = 0.83 °Brix for TSS) and in prediction (R2Pred = 0.75 and RMSEP = 0.01 for MC and R2Pred =0.75 and RMSEP =

1.05 °Brix for TSS) were obtained showing the good capability of the NIR models to accurately predict the MC and TSS in tomatoes samples. Concerning the VIS/NIR models a lower performance was obtained for the optical estimation of MC and TSS in calibration (R2Cal = 0.67 and RMSEC = 0.01 for MC and R2Cal = 0.72 and RMSEC = 1.10 °Brix for TSS) and in prediction (R2Pred = 0.53 and RMSEP = 0.01 for MC and R2Pred = 0.65 and RMSEP = 1.19 °Brix for TSS). However, considering the very low error difference obtained from the models built using NIR and VIS/NIR (from the two different devices) optical data, the use of fewer LVs and less invasive pre-treatments, the prototype has been developed using wavelengths coming from the VIS/NIR optical range (from 450 to 1000 nm). Moreover, this decision was also taken considering the capability of the VIS/NIR optical detectors to be less expensive in order to develop cost-effective optical sensors able to get qualitative information from tomato's samples.

Table 2. Figures of merit of the near-infrared and visible/near-infrared partial least square regression models obtained from the two commercial spectrophotometers for the estimation of moisture content and total soluble solids.

Pre-pro = pre-processing; LVs = latent variables; Cal = calibration; Pred = prediction, TSS = total soluble solids; MC: moisture content; SNV: standard normal variate; VIS = visible/near-infrared; NIR: near-infrared.

						Calibration			Prediction		
Optical range	Qualitative parameter	N° of Cal samples	N° of Pred samples	LVs	Pre-pro	R^2_{Cal}	RMSEC	$Bias_{Cal}$	R^2_{Pred}	RMSEP	$Bias_{Pred}$
NIR (950-1650 nm)	MC (%)	116	80	6	Der 2 + Mean centering	0.82	1	-2.2 e-16	0.75	1	6.2 e-4
	TSS (°Brix)	117		10		0.84	0.83	1.1 e-14	0.75	1.05	-0.2
VIS/NIR (450-950 nm)	MC (%)	114	80	3	SNV + Mean centering	0.67	1	1.1 e-16	0.53	1	2.2 e-3
	TSS (°Brix)	115		3		0.72	1.10	-1.7 e-15	0.65	1.19	-0.24

Sensor readouts exploration and regression

Figure 6 shows the pre-prototype readouts (12 wavelengths, table 1). The data have been handled by merging both sensors in one integrated pre-prototype. However, even though the data do not come from a single sensor, the final reflectance output provide a complete profile which is highly comparable in terms of trend and reflectance of the tomato's samples acquired with the VIS/NIR commercial spectrophotometer.

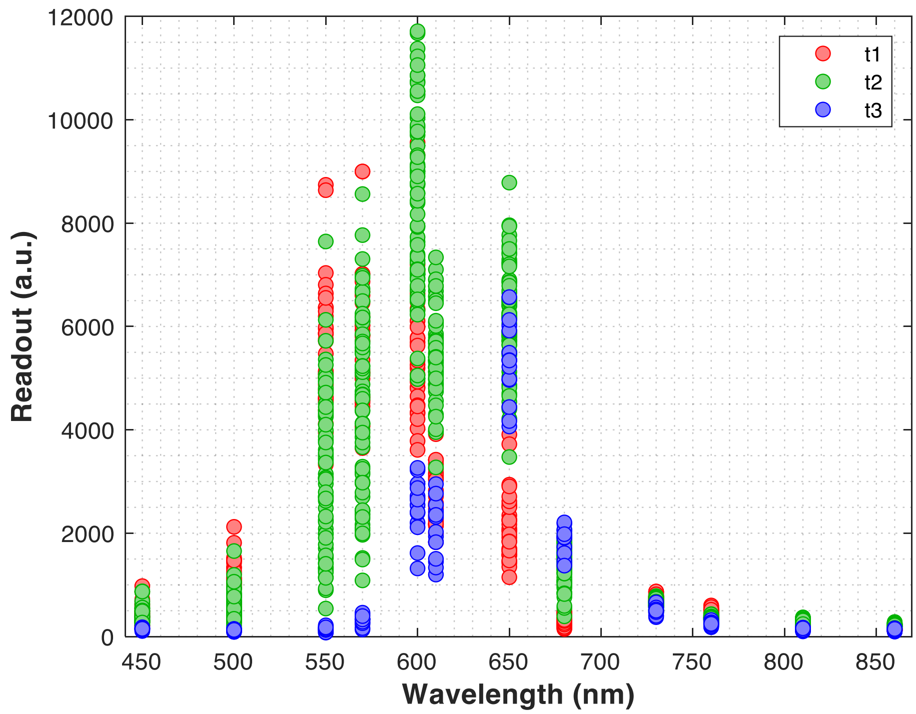

Figure 6. Sensors' readouts obtained from intact tomatoes.

Table 3 shows the figure of merit of the PLS models obtained by the integrated prototype system for the evaluation of MC and TSS. Overall, a pour coefficient of determination was obtained for the prediction of both qualitative parameters (RPred2 = 0.52) using 4 latent variables. However, a slightly higher RMSEP was obtained respect to the model developed using the VIS/NIR spectrophotometer (RMSEP = 0.02 for MC and RMSEP = 1.45 for TSS). These results suggest the capability of the new integrated prototype to be able to obtain better results using more tomato's samples in order to boost the predictive performance of the PLS model.

Table 3. PLS regression calibration statistics for the visible/near infrared regression-models obtained using the prototype spectrometer for the estimation of moisture content (%) and total soluble solids (°Brix).

Pre-pro = pre-processing, LVs = latent variables, Cal = calibration, Pred = prediction; TSS = total soluble solids; MC: moisture content; VIS = visible/near infrared; NIR: near-infrared.

						Calibration			Prediction		
Optical range	Qualitative parameter	N° of Cal samples	N° of Pred samples	LVs	Pre-pro	R^2	RMSEC	Bias	R^2	RMSEP	Bias
VIS/NIR (12 wavelengths)	MC (%)	113	74	4	Autoscaling	0.60	2	-2.2 e-16	0.52	2	-4.5 e-05
	TSS (°Brix)					0.63	1.27	1.7 e-15	0.52	1.45	0.02

Methods comparison

To evaluate whether significant differences of the performance between the VIS/NIR commercial spectrophotometer and the VIS/NIR prototype in determining the MC and TSS exist, the Passing–Bablok regression was performed on the same data used as external validation of the PLS models built using the commercial spectrophotometer and the prototype. Applying a joint test on slopes and intercepts, the devices were compared in pairs analysing the differences between the prediction values for MC and TSS obtained by the models developed using the two instruments. No statistical differences between the instruments were highlighted from the Passing–Bablok tests, at a confidence level of 95%. Therefore, the null hypothesis (slope not significantly different from 1 and intercept not significantly different from 0) was accepted for all the paired comparisons: MC-sensor prediction vs. MC-commercial VIS/NIR spectrophotometer prediction and TSS- sensor prediction vs. TSS-commercial VIS/NIR spectrophotometer prediction. In figure 7, the Passing–Bablok regression lines (solid blue lines) and the bisector of the quadrants (ideal lines) represented as dotted red lines were reported for comparison.

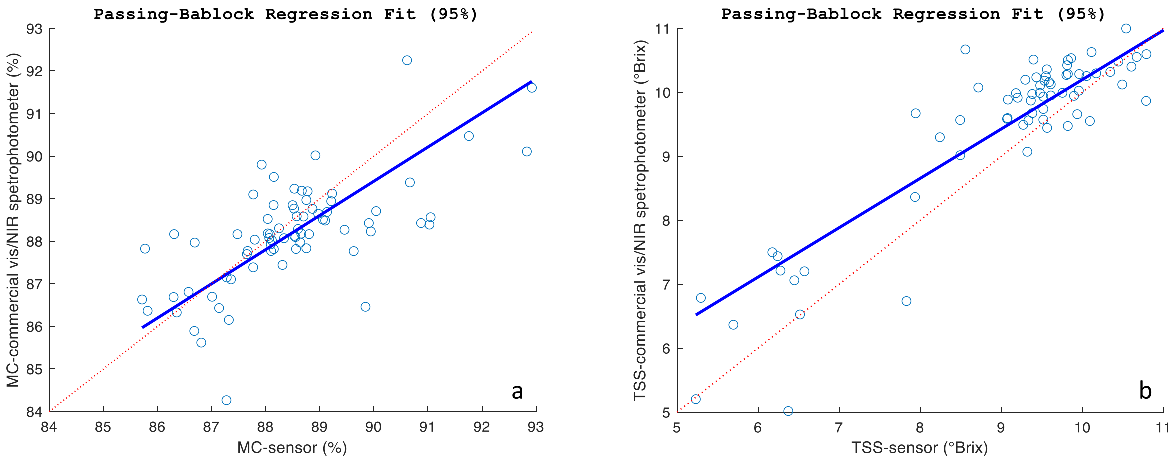

Figure 7. Passing–Bablok regression outcomes: (a) Comparison between sensor and commercial VIS/NIR spectrophotometer for moisture content prediction and (b) comparison between sensor and commercial VIS/NIR spectrophotometer for TSS prediction.

Conclusions

The interest in small-sized, cost-effective and simplified portable devices to quantify quality parameters in a rapid and non-destructive way in pre and post-harvest steps of agri-food chain is desirable. Moreover, the commercial availability of optical components, highly miniaturized, extremely low-cost and robust, given the opportunity to develop an optical device based on LED technology (light emitting diode), which will be tested in this work to monitor the ripening of table tomatoes (Solanum lycopersicum L., Marinda F1).

In this work, a pre-prototype equipped by two visible and SW-NIR sensors for spectral acquisition based on LED technology was tested for rapid estimation of tomato's quality parameters. The overall results of the PLS models from the pre-prototype were encouraging considering the initial development stage of the device. In these terms, a larger dataset in order to improve the robustness of the prediction models is needed. The instrument should be able to acquire and predict moisture content and total soluble solids related to tomato. The integration of simple processing algorithms derived from the PLS models in the microcontroller software would easy calculate and visualize the real-time values of the predicted parameters on the device dashboard. In the envisaged future updated version of the prototype, it is planned that the shape of the device will be able to fully embrace the sample (in this case a tomato, but it can also be used on other vegetable matrices) to minimize and keep under control the incidence of sunlight for in field use.

Moreover, the optical core of the device is a commercial one, as stated, and it is equipped with a standard white LED that showed little power at longer wavelengths. The next evolutional step is to reinforce the lighting system by using one or two additional LEDs with emission peaks around 750 nm and 840 nm.

The potential device deriving from the pre-prototype analysed in this work could result as user-friendly devices to support small-scale growers in determining the optimal harvest date according to tomato ripening degree or applied in post-harvest to classify the fruits based on quality parameters objectively measured. Moreover, the development of interconnected optical systems (creation of remote storage of optical databases) would also allow the updating of the prediction performance of the mathematical models integrated in the prototype for the parameters control. Finally, thanks to the low cost of the components, comparable to consumer electronics, the pre-prototype could be also hypothesized for applications requiring a high number of distributed sensors (sensors network approach), for example, to control agri-food products directly in the field in defined sentinel parcels, or along the supply chains where extreme miniaturization, simplification and low cost can be crucial aspects.

Acknowledgements
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PAPER 2: Application of a cost-effective visible/near infrared optical prototype for the measurement of qualitative parameters of Chardonnay grapes

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Featured Application: The work shows the results of the application of a vis/NIR prototype used to measure qualitative parameters of Chardonnay grapes by building predictive regression models.

Abstract: In this paper, a cost-effective visible/near infrared optical prototype was tested for grape maturity monitoring. The device was used to quantify the qualitative parameters of Chardonnay grapes, based on the combination of spectroscopic data and the creation of predictive models. The optical acquisitions were performed directly in the field through the use of 12 wavelengths in the vis/NIR range, i.e., 450, 500, 550, 570, 600, 610, 650, 680, 730, 760, 810 and 860 nanometers. The prediction of the qualitative parameters was carried out through a multivariate model, partial least square (PLS) regression technique and built knowing the real values of the parameters, i.e., total soluble solids (TSS), titratable acidity (TA) and pH measured through the reference laboratory analyses. Sampling included two harvest years. The most efficient model was the one for TSS evaluation that gave a R_2 = 0.87 (independent test set validation). The results demonstrated that the optical device is able to provide useful information about the ripening parameters of Chardonnay grapes directly in the field in order to predict its correct maturation stage and, therefore, support operators in rapid and objective decision making. Overall, the use of the prototype promotes a sustainable approach and viticulture 4.0.

Keywords: viticulture 4.0; chemometrics; portable; ripeness; field measurement; vis/NIR wavelengths.

1. Introduction

Food products, particularly those with a high value-added, are frequently subjected to stringent quality standards which are critical for attesting to certain specific characteristics such as geographical origin, manufacturing method, and/or producer know-how. Analytical platforms must be updated and improved on a regular basis as fraudulent methods become more sophisticated, as well as the complexity and diversity of food composition [1].

In this context, non-destructive techniques, such as spectroscopic and imaging techniques, are inserted effectively, responding to the problems of the food control field. Since these are analytical techniques that offer numerous advantages, including the non-destruction of samples, the rapidity to obtain results and the possibility of checks during the production processes, they have been studied and exploited for a long time in the agro-food sector [2]. The most commonly widespread non-destructive techniques in the food industry are indeed vis/NIR spectroscopy, NIR spectroscopy (NIRs) and image and multi/hyperspectral analysis [3].

Vis/NIR spectroscopy have been used successfully in non-destructive evaluation of fruits and vegetables quality over the past decades [4] and, in particular, in grape and wine industry e.g. to determine the optimum harvest time or to evaluate quality parameters in works by Kemps et al. [5], Guidetti et al. [6], González-Caballero et al. [7], Giovenzana et al. [8], dos Santos Costa et al. [9], Power et al. [10], Vallone et al. [11], just to name a few.

The possibility of monitoring the ripening stage of the grapes directly in the field has now become of fundamental importance both to guarantee a raw material of the highest quality and to support winemakers in their choices.

Another crucial aspect is covered by sustainability. The chemical analyses normally carried out in the laboratory to evaluate qualitative parameters of grapes require chemical reagents and also a lot of time and specialized personnel; the optical analyses in this case represent a sustainable alternative [12]. Nowadays, commercially available spectrophotometers are mostly expensive benchtop instruments that do not offer the possibility of being used outside the laboratories. The existing portable instruments offer the possibility of being used directly in the field, but also have a high cost and often a configuration that is only useful to acquire spectra and not to obtain immediate results, in terms of parameter prediction. For these reasons, the research is concentrating on maintaining the high performance of this type of optical analysis, while using simplified, portable and easy to use tools [13].

Some papers have been published on the use of portable spectrometers on vegetal matrices. Nagpala et al. [14] used a portable vis/NIR device based on the creation of an index, which was based on two wavelengths peaks (index of absorbance fifference, I_{AD}) to follow the ripening evolution of cherries; Ribera-Fonseca et al. [15] used the same device on grape berries, demonstrating that the use of I_{AD} may be useful for monitoring technological ripeness and anthocyanin concentration. Yang et al. [16] used a portable vis/NIR device implemented with an optical fiber to predict the sugar content (TSS) of kiwi fruits and Fan et al. [17] used a portable vis/NIR device on apples again to predict the content in TSS.

Despite attempts at simplification, these non-destructive and rapid techniques are, however, characterized by an intrinsic complexity of the data collected, which requires multivariate statistical analysis techniques for interpretation. To measure fruit maturity at harvest and during the post-harvest period, various non-destructive techniques and chemometric algorithms have been developed [18].

Chemometrics is a discipline of statistics that deals with multivariate data historically derived from analytical chemistry. The chemometric techniques used to obtain information from these data are identified as pattern recognition techniques and can be divided in turn between unsupervised methods (principal component analysis and cluster analysis) and supervised methods that allow the creation of predictive models, using various techniques including the regression technique.

Exploratory data analysis, such as PCA, is a fundamental step in the analysis of this type of data. It provides an overall view of the system under study, allowing the detection of possible similarities/dissimilarities among samples, the identification of clusters or systematic trends, the discovery of those variables that are relevant to describe the system and that can be discarded in principle, and the detection of possible outlying, anomalous, or at the very least suspicious samples [19]. The proper management of outliers is necessary to develop models that are effective [20]. Regression models can therefore be used to predict and quantify selected maturity indices of products. Chemometric techniques are, therefore, fundamental for extracting useful information from optical data and for creating simplified models. In this case, a PLS (partial least square) regression model was used, which represents a particular type of multivariate analysis that is capable of modeling the relationship between two matrices, i.e., the relationship between the predictors, X, and the variables we want to predict, Y [21].

The experimentation tested a cost-effective and portable prototype to estimate the qualitative parameters of Chardonnay grapes directly in the field in order to support operators in rapid and objective decision making. The aim of this work is to obtain immediate results on grape ripeness to identify the optimal harvesting time. The optical prototype is equipped with vis/NIR bands and predictive models to promote a sustainable approach and viticulture 4.0.

2. Materials and Methods

2.1. Sampling activities

The experimental campaign took place in the viticulture area of the province of Mantua at "Azienda Agricola Ricchi" (Monzanbano, Lombardy, Italy; 45° 23' 22.848" N 10° 41' 36.427" E) during the ripening period among the end of July and the end of August 2020 and 2021. Sampling was performed on Chardonnay grape (*Vitis vinifera* L.) in different plots distributed in the vineyard. Chardonnay is a white grape strong variety with a moderate grape production; its berry clusters are tiny, cylindrical, and winged, and can range in size from well-filled to compact [11].

All the samples were collected from 8 plots and in each plot, 3 plants were selected and marked in order to take the samples as representative as possible of the entire vineyard. In 2020, a total of 80 samples were collected and analyzed in 4 different sampling dates (8 samples for time 0, 24 samples for each of the other 3 sampling dates). In 2021, a total of 96 samples (24 samples for each sampling date) were collected with the same procedure.

2.2. Optical analysis and prototype features

The acquisitions were performed directly in the field (immediately after manually collecting the samples), without any sample preparation. For each sample, 5 single berries were analyzed to obtain a total dataset of 400 optical analyses in 2020, corresponding to 40 acquisitions for time 0 and 120 acquisitions for the other samplings. In 2021, 480 optical analyses (corresponding to 120 acquisitions per sampling date) were collected and made up the dataset.

A first version of the prototype was described in 2021 [22] as a pre-prototype; the device was used for optical acquisitions during 2020 sampling. The new version of the device used for 2021 sampling consists of 2 optical sensors with 6 wavelengths each, for a total of 12 wavelengths, as described in Table 1.

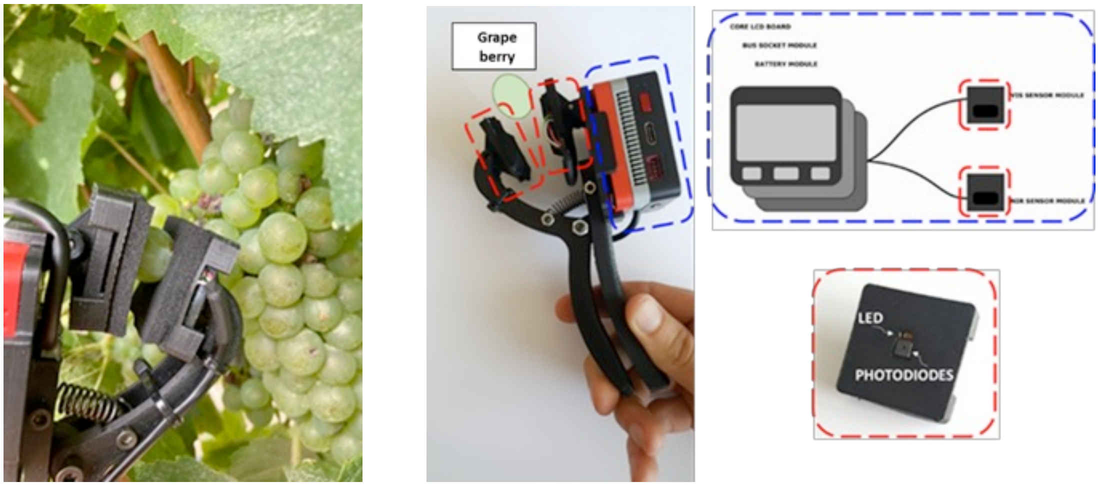
Table 4. Device wavelengths.

	Wavelengths (nm)					
Sensor 1	450	500	550	570	600	650
Sensor 2	610	680	730	760	810	860

The prototype incorporates the two sensors just mentioned (AMS, models AS7262 visible and AS7263 NIR, Premstaetten, Austria, Europe) for spectral acquisitions in visible (vis) and in short wave near-infrared (SW-NIR) regions. The sensors have a 16-bit radiometric resolution and 12 independent on-device optical filters, from 450 to 860 nanometers. The full-width half-maximum of vis sensor is 40 nm, while the full-width half-maximum of SW-NIR sensor is 20 nm. The use of these commercial optical cores allows the cost of the prototype to be kept extremely low.

The wavelengths used in the device are the same as in the pre-prototypal version [22], precisely because each wavelength corresponds to a particular absorption peak of particular interest in this type of analysis. As reported by Giovenzana et al. [8], 630 and 690 nanometers are near the characteristic peak of chlorophyll, 730 nanometers is near the third overtone of the -OH bond, and lastly, 810 and 860 nanometers are near the combination band of the -OH groups of sugars.

Unlike the first version of the device, now the two sensors are integrated into a clamp that enables the operator to fully embrace the berry grape (Figure 1a, b) and capture all the readouts at once, without the need to repeat the analysis with the first sensor and then with the second sensor. Moreover, the instrument was configured to perform an average of 10 scans for each acquisition, trying to reduce as much as possible the experimental noise associated with the light which often creates issues with optical analyses.



(a) (b)

Figure 8. (a) Prototype clamp wrapping the sample; (b) prototype layout.

2.3. Chemical analyses

The wet-chem analyses were carried out on the same samples on which the optical data were collected and were used as reference parameters. In particular, three characteristics parameters generally used for estimating grape's technological maturity were measured. Total soluble solids (TSS) were measured using a digital refractometer (PAL-1 ATAGO, Tokyo, Japan, accuracy refractive index ± 0.2 Brix), which provides the result in Brix, measuring the refractive index of the juice obtained from mashing of the sample according to the total content of soluble solids. Titratable acidity (TA) was measured on the juice with an automatic titrator (TitroMatic KF 1S, Crison Instruments, Milan, Italy) and the result was expressed in grams of tartaric acid per liter ($g_{tartaric\ acid}$ dm^{-3}). The pH was measured on the juice using a portable pH meter PCE-PHD 1 (PCE Inst. GmbH, Meschede, Germany).

2.4. Data processing

The results of chemical reference parameters (TSS, TA and pH) and the optical data obtained by the device were analyzed in Matlab® environment, version 2021a (MathWorks, Inc., Natick, MA, USA) using PLSToolbox package (Eigenvector Research, Inc., Manson, WA, USA).

The first part of data processing involved the creation of matrices showing the optical data and the execution of a first Principal Components Analysis (PCA). This first unsupervised exploratory analysis allowed the identification, and then removal, of some outliers using the "Hotelling T2 computation" function. The data matrix used was averaged and then reduced to obtain a new matrix (similarly representative of the sampling campaign) made up of 24 samples for each sampling date (except only for the time zero of 2020 which consists of 8 samples as mentioned above) switching from a matrix with 880 rows to a matrix with 176 rows.
After performing the exploratory PCA analysis and removing outliers, reference parameters (TSS, pH and TA) were used for the calculation of three different predictive models. The partial least square (PLS) regression technique was used to create the models, with only autoscaling as pretreatment. Cross-validation was used with the leave more out technique, venetian blinds with 5 cancellation groups. Concerning test-set validation, the dataset was randomly divided into a training set, corresponding to the calibration set, and into a test set. A total of 60% of the samples were used for the training set and 40% for the test set. To evaluate model accuracy, root mean square error (RMSE) and coefficient of determination (R^2) were used; the better the model performs, the lower the error is, and the higher the R^2 (as maximum equal to 1) is.
In addition, the ratio between the standard deviation of the response variable and RMSE (RPD) was calculated. An RPD between 1.5 and 2 means that the model can discriminate low from high values of the response variable; a value between 2 and 2.5 indicates that coarse quantitative predictions are possible, and a value between 2.5 and 3 or above corresponds to good and excellent prediction accuracy, respectively [23].
Finally, a significance test (Student's t-test) was performed between refence and predicted data along the ripening evolution monitoring for each year considered.

3. Results

3.1. Reference parameters (TSS, pH, TA)

In Figure 2, the descriptive statistics of the parameters of technological maturation measured in the laboratory are reported. The data are divided into the four sampling dates and the mean, the median, the interquartile range and the data range are represented in the figure. The graphs also show the outliers and extreme outliers. The outliers highlighted are the values that are more than 1.5 times the interquartile range away from the top or bottom of the box. The characteristic trends of grape ripening are clearly visible from left to right; the total soluble solids and the pH increase, while the titratable acidity tends to decrease as the maturation progresses [24].

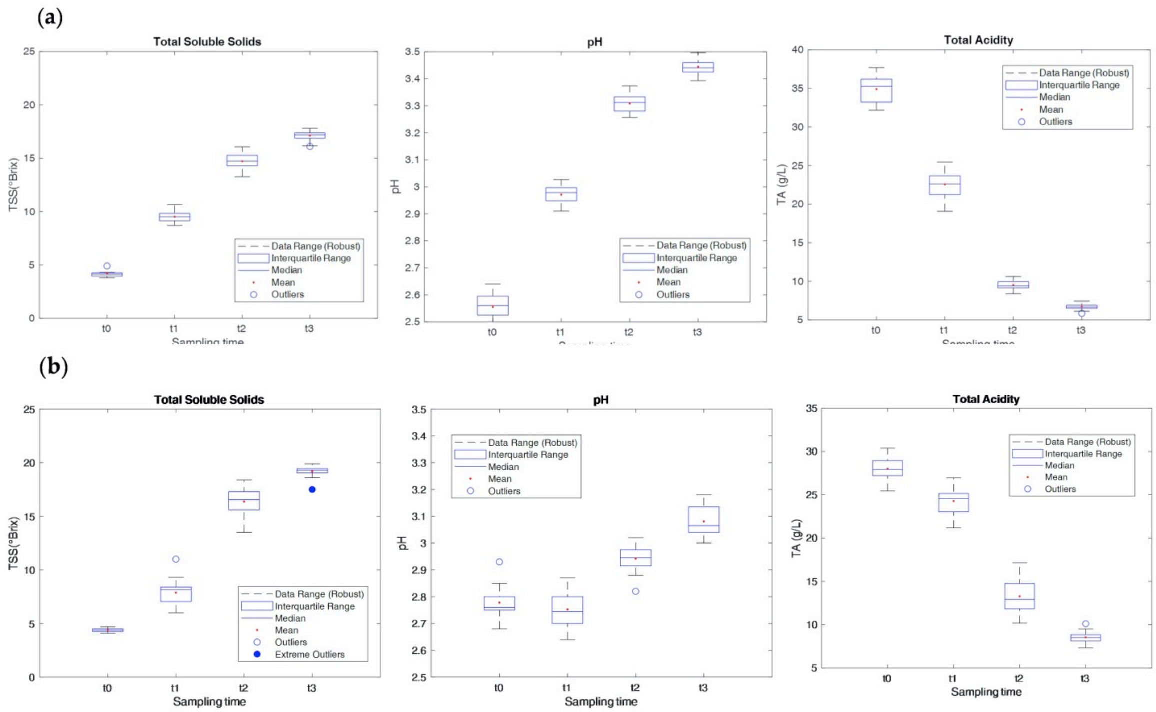


Figure 9. Box plots reporting descriptive statistics of reference parameters (TSS, pH, TA) in 2020 (a) and 2021 (b).

The trend of the qualitative parameters clearly shows the same trend in the year 2020 and in the year 2021. The 2020 data (Figure 2a) are characterized by higher variability between the dates than the 2021 sampling (Figure 2b), while the 2021 data highlight higher variability within the dates, with respect to the 2020 sampling.

3.2. Optical readouts

Figure 3 shows the total readouts of both sampling years, colored according to the qualitative parameters. Data from the two different sources, i.e., the pre-prototype [22] and the new version of the device and the two different years, were merged to observe the sample reflectance in the whole optical range covered by the sensors. Even if the device is not able to provide continuous spectra, the increasing and decreasing trends of the measured parameters are visible by observing the readouts in their entirety.

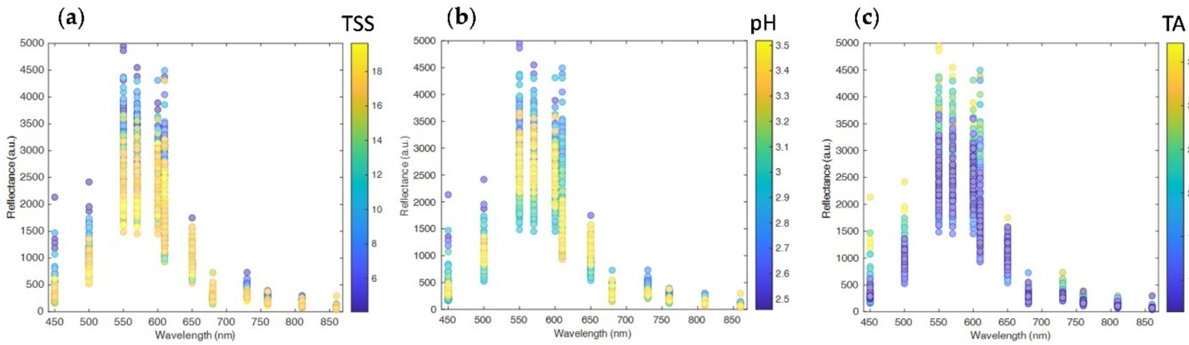


Figure 10. Total readouts of four sampling dates of both sampling years, labelled according to the reference parameters: TSS (a), pH (b) and TA (c).

The readouts represented in figure 3a are colored based on total soluble solids, the readouts in figure 3b are colored based on pH, while the readouts in figure 3c are colored based on titratable acidity. The progressive ripening trend determines the color variations in the samples, which are strictly related to the ripening evolution. Variations in green color occur; initially, it is very bright then it tends to turn gradually towards light green, almost yellow. The decreasing trend of the optical outputs is due to high reflectance values corresponding to the less ripe (and therefore greener) grapes; as the ripening progresses, the reflectance values of the samples tend to decrease [25]. Evidently, these effects are more noticeable in the visible region readouts (460-650 nm).

3.3. Principal Component Analysis

As already discussed in Section 2.4, principal component analysis constitutes a fundamental part of chemometric data processing. Figure 4a shows on the x axis the first principal component (PC1), which explains 42.97% of the variance and on the y axis, the third principal component (PC3), which explains 9.78% of the variance. In the orthogonal space defined by the two components (which account for about 53% of the original variance), a horizontal trend is evident along the PC1, which could be attributable to the maturation of the samples and the variations that occur in berries over the course of time, mainly evolution of skin pigmentation. The ripening trend is underlined by the labelling of the samples according to the content of soluble solids (TSS); from right to left, we observe the same trend also recognizable in the total readouts of the device.

Therefore, the interpretation of scores plot suggests that the main source of variability is the progress of maturation. To fully understand the results of the PCA, it is also necessary to observe the loadings plots, as reported in Figure 4b, c. The second principal component (PC2) seems to be attributable to the use of two different sensors. PC2 is positive for the wavelengths of the first sensor (450, 500, 550, 570, 600 and 650 nm) and negative for the wavelengths of the second sensor (610, 680, 730, 760, 810, and 860 nm). This principal component could explain the information related to the acquisition methodology of sensors operating in two different ranges of the electromagnetic spectrum, i.e., visible and NIR, respectively.

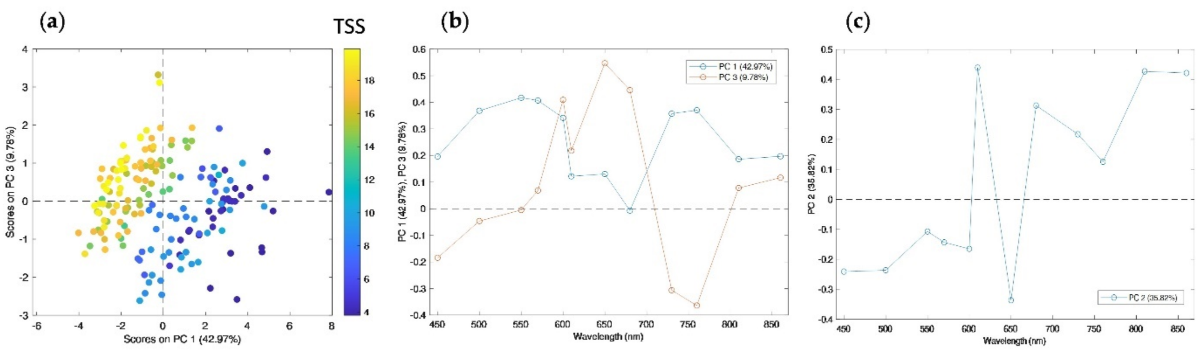


Figure 11. (a) Scores plot of PC1 and PC3, labelled according to TSS content and relative loadings plot; (b,c) loadings plot of PC2.

3.4. Predictive Models

The partial least square (PLS) regression method was used to build predictive models using 2020 and 2021 datasets together to try to better verify the predictive capability. The only pre-treatment that was performed on the data was autoscaling; by dividing the variables into their respective standard deviations, it is possible to give each variable the same importance by imposing equal weights in the analysis [26,27].

The prediction capability of the PLS models was verified by calculating the parameters to evaluate it, both in cross-validation and in prediction. Table 2 shows the figure of merit of the three predictive models built by considering two latent variables (LVs).

*Table 5. Figure of merit of the Partial Least Square (PLS) models calculated with two Latent Variables using optical data pretreated with autoscaling. *SD = standard deviation of reference parameters; R2CV = coefficient of determination in cross-validation; RMSECV = root mean square error of cross-validation; R2Pred = coefficient of determination in prediction; RMSEP = root mean square error in prediction.*

Parameter	LVs	Treatment	SD*	R2CV	RMSECV	R2Pred	RMSEP	RPD
TSS	2	autoscaling	5.35	0.88	1.84	0.87	1.90	2.81
pH	2	autoscaling	0.26	0.56	0.18	0.62	0.14	1.85
TA	2	autoscaling	8.85	0.83	3.59	0.80	3.94	2.25

The most efficient model is the model for TSS prediction (R^2 in cross-validation = 0.88 and in prediction = 0.87) and secondary, the model for TA prediction (R^2 in cross-validation = 0.83 and in prediction = 0.80). The least performing model is the one built for the prediction of the pH with a R^2 in cross-validation of 0.56 and in prediction of 0.62. Maturation curves, which represent the measured and predicted (through the use of PLS models) values for each qualitative parameter, were created. For the creation of the curves, the average values for each sampling date were calculated to be compared with the average values obtained using the device. The maturation curves that represent the 2020 data show more evident differences between the reference values measured by wet-chem analyses (in red color) and the values predicted by the model (in blue color). The performance improvement of the models for the prediction of the three qualitative parameters is to be attributed to the innovative structure of the prototype, which, as already mentioned, has been modified with respect to the original device in pre-prototype form that did not have the two sensors integrated in a single instrument. The integration of the two sensors in a single hardware allowed for better performance in terms of efficiency of light transfer, both from the LED to the agri-food product and from the grapes to the photodiode, i.e., a better signal to noise ratio [28]. Table 3 shows the Student's t-test results performed on the values used to build the maturation curves reported in Figure 5.

*Table 6. Student's t-test performed between refence and predicted data for each sampling time (t0-t3) and for each year considered (2020 and 2021). n.s. = difference not significative at p < 0.05; * = difference significative at p < 0.05; ** = difference significative at p < 0.01; *** = difference significative at p < 0.001.*

Time	TSS		TA		pH	
	2020	2021	2020	2021	2020	2021
t0	*	***	**	n.s.	***	n.s.
t1	n.s.	n.s.	*	n.s.	n.s.	n.s.
t2	n.s.	n.s.	*	n.s.	n.s.	n.s.
t3	***	***	***	n.s.	***	n.s.

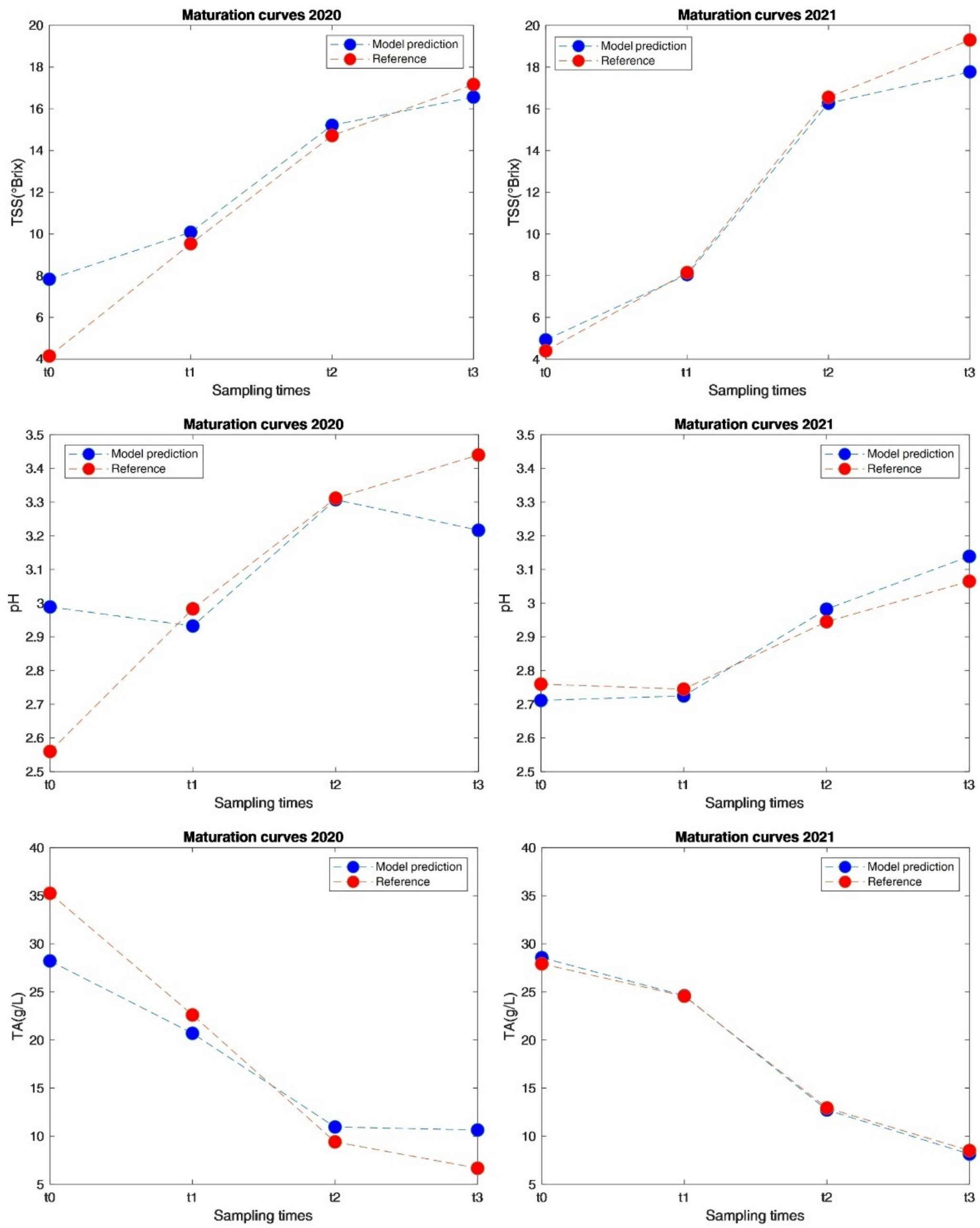


Figure 12. Maturation curves of the two harvest years (2020 and 2021) of the three qualitative parameters: in blue, the average values predicted by the models and in red, the average values measured by wet-chem analyses.

The test carried out a comparison between the values of the qualitative parameters measured in the laboratory (reference parameters) and the values predicted by the device, i.e., the values obtained from the PLS predictive models. The values were compared for each qualitative parameter and for both sampling years, and for each sampling time. The results demonstrated that the integrated version of the prototype (2021) provides more accurate quality parameters estimation than the pre-prototype version used in 2020 sampling. This phenomenon is highlighted by the non-significative differences (n.s.) in the 2021 comparisons, e.g., for TA and pH, no sampling time shows differences between the reference and predicted values.

4. Discussion

The sampling turned out to be representative; an improvement to obtain an even greater variability could be to carry out sampling at constant days apart to collect data with a more constant variability, in particular in the central week of August. With regard to the models' predictive capabilities, further experiments are needed to test the device performance in real operative conditions, both on Chardonnay and on other cultivars. The low variability between dates and the high variability within dates in 2021 sampling, compared to 2020 data, could have led to a worst result in the application of predictive PLS models in the estimation of the three parameters (Figure 5) for the year 2021. Instead, the new and more efficient version of the prototype allowed for better performance in the application of the PLS model for 2021 sampling, as also confirmed by the Student's t-test results reported in Table 3. The creation of predictive models will certainly help the development of simpler vis/NIR prototypes that can be applied directly in the field, making non-expert personnel able to estimate the quality of agri-food products in an objective, instant and more sustainable way.

This work has focused on the applicability of the optical and cost-effective prototype in the wine sector, to define the grape quality by better identifying the exact moment of ripening and obtaining high quality wine. The tested prototype could be easily applied to other small fruits, such as olives and blueberries (after an appropriate modelling phase), for qualitative characterization. Moreover, it could be used at different points of the supply chain, both in pre-harvest to define the best harvest period directly in the field, but also in post-harvest for fruit selection to create different quality classes, optimizing the production process and reducing waste. Moreover, optical devices have been recognized as a green technology capable of estimating quality parameters, producing a low environmental damage compared to traditional wet-chem analyses [12]. Applying smart devices, such as the prototype developed and tested in this work, would help the sector to obtain a sustainable supply chain from an environmental point of view and in line with the industry 4.0 approach [29].

In order to summarize the strengths, weaknesses, opportunities, and threats related to the application of the experimental outputs and concepts of this work in the agri-food sector, a SWOT table was created (Table 4).

Table 7. Strengths, weaknesses, opportunities, and threats related to the application of the experimental outputs and concepts of this work in the agri-food sector.

	Strengths	**Weaknesses**
Internal	Selection of vis/NIR bands easily available on the market Real-time monitoring quality parameters of agri-food products in an objective way, directly in field or in post-harvest conditions Cost-effective of the smart device Remote control devices	Need to control environmental conditions during optical acquisitions High variability of agri-food products Research efforts in optimizing quality parameters estimation
	Opportunities	**Threats**
External	Optimization of agri-food chains Better management of agri-food products Waste reduction Lower environmental impact of agri-food chains Suitable also for SME	Strong link with traditional methods by operators Reduced orientation towards innovation by operators, still managed by old generation

5. Patent

Patent application PCT n. PCT/IB2022/051110 (8 February 2022) for Portable Device for Analysing Vegetable Matrices on the Field and Related System and Method.

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4.2 THE SUSTAINABILITY OF SPECTROSCOPIC ANALYSES

Several features of vis/NIR technology render it a sustainable technique or at least more sustainable than current laboratory analyses used for measuring several qualitative parameters of food (Beć & Huck, 2019).

It has become crucial to employ increasingly green technologies that consume less energy and fewer solvents throughout the entire food production chain, thereby offering advantages in terms of environmental sustainability and beyond (Feliciano et al., 2022).

These non-destructive optical methods are rapid and user-friendly, providing a means to objectively measure qualitative parameters of various food matrices without any sample preparation and without requiring specialized personnel to operate the instruments (Nicolai et al., 2007; Cozzolino, 2022). The absence of solvents makes these techniques not only more sustainable but also safer for operators (De La Guardia & Armenta, 2010).

When comparing optical analyses with traditional laboratory analyses, the latter are significantly more time-consuming and expensive, requiring a longer and more complex sample preparation and the use of chemical reagents (Cozzolino, 2019). To quantify the sustainability advantage, a Life Cycle Assessment (LCA) study was employed. LCA is a tool capable of analyzing the environmental impacts associated with the different stages of a product's or service's life cycle (Casson et al., 2023).

In the following paper, wet-chem analyses for measuring three technological parameters of grapes are compared with two different optical instruments from an environmental point of view: the final version of the prototype described in section 4.1 (paper 2) and a benchtop instrument.

PAPER 3: Visible/near-infrared spectroscopy devices and wet-chem analyses for grapes (Vitis vinifera L.) quality assessment: an environmental performance comparison

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Abstract

Background and Aims. The composition of grapes (Vitis vinifera L.) at harvest is a key factor that determines the future quality of wine. The work aimed to evaluate and compare the environmental impact of the most evaluated technological parameters using three approaches: the wet-chem method, the optical method using benchtop devices, and the optical method using a prototype smart and cost-effective device (Technology Readiness Level: 5). *Methods and Results.* The life cycle assessment (LCA) methodology was used to identify the most environmentally sustainable solution in a "from-cradle-to-grave" approach. The functional unit was identified by the execution of the analyses necessary to measure the three technological parameters: TSS, pH, and TA. The findings show that the optical analysis carried out with the prototype is the most suitable and green solution (i.e., with the lowest environmental impact in all the impact categories analysed); in fact, the same technology has proved to be 3.2 times more sustainable than the wet-chem method for the best average environmental performance. *Conclusions.* The research demonstrated the environmental impact advantages of the optical analyses for assessment of grape composition. *Significance of the Study.* Innovations in agriculture and the development of smart solutions represent advantages for managing and monitoring the composition of agri-food products that are a green solution for the industry.

Keywords: chemometrics, LCA, optical techniques, prototype, sustainability.

1. Introduction

Grape composition at harvest is a key factor that determines the future quality of wine [1, 2]. By measuring certain grape parameters, such as TSS, reducing sugar, tartaric acid and malic acid, and TA and pH value, it is possible to determine the optimum harvest timing to ensure the production of high quality wines [3]. These parameters are usually obtained through wet-chem analyses, which often require samples to be sent to geographically distant laboratories that can take a long time to return results. These types of analyses have proved to be slow, time-consuming, and destructive, require expert qualified personnel, and do not respond to the demand of the modern wine industry [2, 4]. By considering more efficient analytical technologies compared to the traditional ones, wineries will increase their efficiency along the production chain [5].

Generally, wine chain production is the responsibility of the oenologist. This professional aims to manage and control all the production processes in the field, starting from the choice of the appropriate cultivar to be planted in the vineyard to the identification of the optimal time for harvesting, and in the winery through the vinification process and finally bottling. The oenologist is also essential to evaluate the composition of the grape through the chemical analyses cited above, with such information being valuable during the decision making to optimise productivity [6]. Even though this role is important because of its professional experience, the modern wine industry cannot rely on this approach alone but needs more rapid analyses. To satisfy this need, innovative technologies are evolving. In the agri-food industry, spectrophotometers can perform rapid analysis of key composition parameters, giving a precise evaluation of composition. These devices can be used to obtain results in less than 3 min, without using chemical reagents and without having additional costs, even though it requires sample preparation through purification and degasification. In fact, spectroscopy can be used to obtain the chemical composition of a biological product, avoiding destructiveness and slowness. The VIS/NIR spectroscopy consists of the combination of effective sensors with sophisticated mathematical models and computational algorithms to establish relations between selected chemical properties and quality attributes [7]. Essentially, with VIS/NIR technology, the sample is illuminated by light radiation absorbing a certain amount of it and reflecting the remaining part at specific wavelengths. The quantity of light reflected and transmitted is traduced into a spectrum by the VIS/NIR detector [8]. The results obtained can be correlated to chemical parameters using chemometric techniques.

In the past, VIS/NIR technology has been used for the determination of the composition of many agricultural products both at a laboratory scale and on-line [9]. The advantages of this technology can also be brought to the wine industry representing a considerable benefit to the sector at different levels: first, by monitoring the composition of the grapes during their growth, it is possible to achieve better management of field practices (e.g., use of water during irrigation) and create a model to monitor the composition of grapes to identify the best harvesting time. Another advantage involves the harvest period during which this technology can be used to identify and separate grapes of different composition so that the value of the final product can be improved [10]. The results derived from VIS/NIR spectroscopy are comparable with those obtained with traditional analytical methods [11–13], with the only difference being that VIS/NIR does not require sample preparation and chemical reagents.

The determination of the composition of incoming grapes in the grading area is also a fundamental step for the overall evaluation of grapes that every winery or grower association needs to consider during winemaking. More than the analysis itself, sampling is the most critical operation, since it is here that the sample, analysed to assess the composition of the entire batch, is obtained and needs to be a representative of the entire load. Nowadays, in the wine sector, truck or bin sampling systems can be used to collect samples with a motorised probe picking up the grapes and crushing them; must is obtained and submitted to analysis to measure main parameters of composition, such as TSS and TA, with a maximum duration of 3 min per truck. Despite that, this type of analysis is defined as destructive due to the fact that the samples analysed are not preserved.

With the shift from conventional to precision agriculture, there are many other proposals on the market to replace wet-chem laboratory instruments, such as simplified portable systems which use a set of discrete commercial modules, such as LED arrays, white light sources, photodiodes, filters, and microspectrometers. The technology is undergoing rapid development and is moving towards miniaturisation. The consumer electronics industry is driving the convergence of digital circuitry, wireless transceivers, and microelectronic-mechanical systems (MEMS), which makes it possible to integrate sensing, data processing, wireless communication, and power supply into low-costmillimetre-scale devices [14]. The resulting miniaturisation and cost reduction of electronic components are creating a completely new method of data acquisition and management using wireless sensor networks (WSNs) based on small battery-powered nodes. A WSN consists of small and low-cost Internet of Things (IoT) devices in a network of peripheral nodes. The nodes are equipped with sensors and a wireless module for data transmission to an online database, where the data are stored and accessible to the end-user. The nodes are energy independent and are installed in areas which are more representative of the vineyard variability [15]. The WSN technology is widely used to monitor environmental factors, such as temperature, moisture, RH, and leaf wetness, which are essential for the decision making of growers [16]. The availability of miniaturised optical devices, however, is driving the research to develop IoT sensors which are highly sensitive for the detection of substances in an environment, such as chemicals or biological materials. In viticulture, the sensors are directly installed in proximity of the target (vine leaf or grape bunch) to remotely monitor the vineyard during the crop season. These instruments provide a wide range of information, but they require a human operator for data acquisition and are not standalone. For future scenarios, they will need to be optimized bringing the laboratory directly into the vineyard, without human intervention for data sampling [17].

Casson et al. [18] demonstrated that VIS/NIR technology can be defined as a sustainable solution to monitor the composition of olives and that the analyses carried out to monitor the composition of grapes are different; as a result, they considered that the issue of the sustainability of the technology in the wine sector remains to be determined. Overall, innovation in agriculture activities tends to propose smart solutions that can represent advantages of managing and monitoring agri-food product composition, but the sustainability level of these solutions remains a knowledge gap. This work aims to evaluate and compare the environmental impact of three scenarios based on two different methods, chemical and optical, used in the wine sector to measure the main composition parameters of grapes: TSS, TA, and pH value. For each of these parameters, chemical methods and VIS/NIR spectroscopy, located in benchtop instrumentation and using an innovative smart solution (optical in-field prototype), will be analysed and then compared. The life cycle assessment (LCA) methodology and estimation performance were carried out to identify the most sustainable solution and to propose actions to reduce waste and the impact along the wine supply chain.

2. Materials and Methods

The basic idea of LCA is that all the environmental burdens connected with a product or service must be assessed, back to the raw materials and through to waste removal. This method is developed according to the international standard ISO 14040-14044 [19, 20].

2.1. Goal and Scope Definition

The goal of this study was to evaluate and compare the environmental impact of two types of method, chemical and optical, used in the wine sector to measure the composition of grapes. Three main quality parameters were considered: TSS, determined by a digital refractometer; TA, analysed by volumetric titration; and pH value measured with a pH meter [21]. The definition of the environmental impact was performed for both the chemical methods (destructive analyses) and the VIS-NIR spectroscopy (nondestructive analysis).

Specifically, for the nondestructive analyses, two technologies were considered: benchtop spectroscopy and the prototype of a simplified and portable device which incorporates sensors and is used to measure the same three compositional parameters cited above. Such a technology, compared with the benchtop, can be used directly in the field and allow the creation of a measurement database based on a cloud system [17].

2.1.1. Functional Unit. According to the ISO 14040 standard, the functional unit represents the performance of the outputs of a product system providing a reference to which inputs and outputs are related [19, 20]. In this comparative study, the functional unit was identified by the pool of analyses carried out for the three different parameters, that is, three chemical analyses for the wet-chem analyses and one single nondestructive analysis for the optical analysis to estimate the three grape composition parameters.

2.1.2. Definition of the System. The system under study included all the procedures related to the chemical analysis, traditionally used in laboratories, and the VIS/NIR technology. For the former, all activities necessary to obtain three replicates for the three parameters were considered. The latter consisted of one single optical measure carried out three times to obtain results for the three parameters simultaneously. All the inputs and outputs necessary to complete the study were collected through some interviews at the laboratory of the Università degli Studi di Milano, which specializes in analysis of grape composition. The instruments are used only for this purpose; during other periods of the year, the instruments are not used. Considering this usage pattern, the laboratory capacity should be defined as equal to 450 analyses/year, defining the average number of analyses carried out in a day equal to five.

2.1.3. System Boundaries. The system boundaries determine which unit processes shall be included within the LCA (Figure 1). In this study, a "cradle to grave" approach was used, which considers all the inputs of the process: from the extraction of the raw materials, through the construction of the laboratory materials and analytical tools, the chemicals and the calibration of the vis/NIR technology including energy, electricity and water supply. In addition to that, the system boundaries considered the outputs of the process, thus the disposal of every single material used during the analysis (exhausted plastic, paper, chemicals and sample). The laboratory carried out the wet-chem analyses for 3 months during the maturation period (July to September), and all the tools were not used for other tasks or in another period. Instead, the vis/NIR technology was considered as an alternative method to measure the same quality parameters but without the destructiveness of the sample.

2.2. Life Cycle Inventory (LCI)

Inventory analysis involves data collection and calculation procedures to quantify relevant inputs and outputs of a product system [20]. All the inputs and outputs collected in the study should be interpreted, depending on the goals and scope of the LCA. The data within LCI also constitute the starting point to the life cycle impact assessment.

For this study, the information necessary to the inventory phase should be referred to the methods of the Organisation Internationale de la Vigne et du Vin (OIV); despite that, the data collection of each analysis was obtained through interviews carried out with the laboratory of the Università degli Studi di Milano. All the materials used and described in the interviews were analyzed: from analytical tools and laboratory materials up to reagents, the quantity of the sample and energy, in addition to all the waste generated along the procedures. For the LCI, it was also necessary to indicate the lifetime of the analytical tools and the laboratory materials expressed in years, in addition to the number of analyses carried out by each machine during their lifetime. All the analytical tools have a lifetime of 15 years with a total of 6750 analyses while the laboratory materials last 10 years with 4500 complete analyses. For the present study, the inventory of every single analysis was performed separately and reported.

2.2.1. Allocation Procedures. According to the International Organization for Standardization [19], allocation is the tool for partitioning the input/output flows of a process or a product system between the product system under study and one or more other product systems.

As a matter of fact, during laboratory analyses, many analytical tools or laboratory materials are used for more than one type of analysis (i.e., computer, automatic volumetric titrator and pipettes), so it was necessary to adopt an allocation procedure which consisted in allocating all the inputs of every chemical and optical analyses (benchtop and prototype) to a specific value, as reported in a previous study [22]. This value is known as the amount per analysis and requires different variables to calculate it: the allocation factor (Af) ranging from 0 to 1, the quantity of input (Q) in terms of mass, volume, energy, and the number of analyses (Noa) performed during the lifetime of the product under study.

$$Amount\ per\ analysis = \frac{Q * Af}{Noa}$$

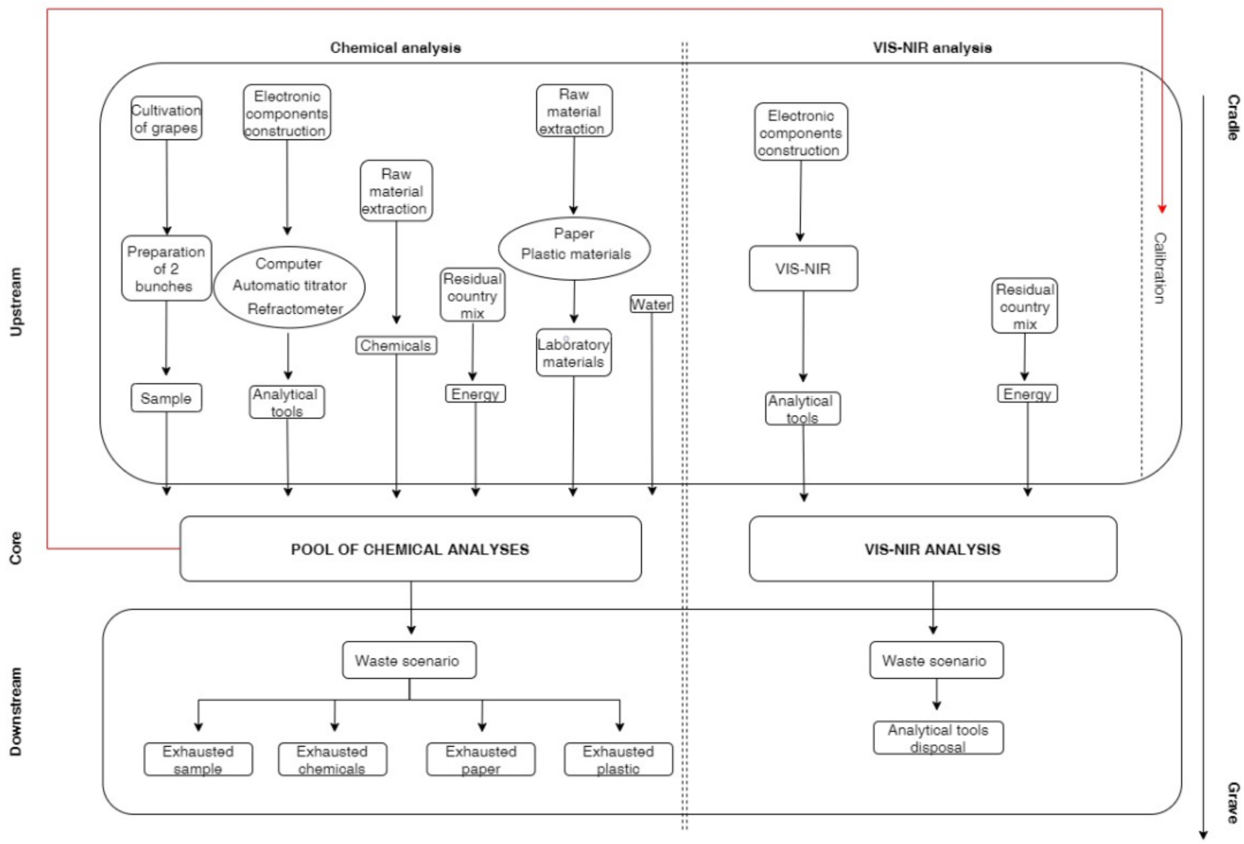

*Figure 1. System boundaries and elements flow. *Calibration = chemical analysis + benchtop analysis/prototype analysis.*

2.2.2. Preparation of the sample LCI. For the preparation of the sample used in the chemical analysis, two bunches of grapes were collected from field and squeezed manually within a plastic bag (20x30 cm) to obtain 100 g of must. A solution of 0.2% sodium azide (NaN_3) (1mL) was added to avoid fermentation and alterations in must and to guarantee storage of the sample for long periods (weeks or even months). For this study, must was stored at room temperature for 2 weeks until analysed in the laboratory. Input and output data related to this procedure are reported in Table 1.

Table 1. Quantity, allocation factor, lifetime and amount per analysis for the preparation of the sample.

	Unit	Quantity (Q)	Allocation factor (Af)	Lifetime (years)	Lifetime (No. analyses)	Amount per analysis
Input						
Must	g	100	0.33	n.a.	1	33.333
Plastic container (100 mL)	g	15.91	0.33	10	4500	0.001
Plastic tops	g	4.06	0.33	10	4500	0.000
Plastic bags (20x30)	g	6.57	0.33	n.a.	1	2.190
Sodium azide (0.2%)	g	1	0.33	n.a.	1	0.333
Paper towel (for materials cleaning)	g	2.03	0.33	n.a.	1	0.677
Deionised water (for materials washing)	g	5	0.33	n.a.	1	1.667
Manual pipettes (100–1000 μL)	g	83.42	0.33	10	4500	0.006
Pipette caps (100–1000 μL)	g	0.787	0.33	1	1	0.262
Output						
Sample waste	g	230	n.a.	n.a.	n.a.	n.a.
Plastic waste	g	7.36	n.a.	n.a.	n.a.	n.a.
Paper waste	g	2.03	n.a.	n.a.	n.a.	n.a.

2.2.3. Total acidity determination LCI. Total acidity, expressed in g/L, was determined with an automatic volumetric titrator, associated with an autosampler and a computer. The automatic volumetric titrator used 0.1 N sodium hydroxide as the titrant. Starting from must, 7.5 mL was collected, and 50 mL of deionized water was added to start the analysis with the autosampler and titrator. All the inputs and outputs related to this analysis are summarized in Table 2.

Table 2. Quantity, allocation factor, lifetime and amount per analysis for the TA determination.

	Unit	Quantity (Q)	Allocation factor (Af)	Lifetime (years)	Lifetime (No. analyses)	Amount per analysis
Input						
Must	g	7.5	1.00	1	1	7.500
Automatic volumetric titrator	g	20×10^3	0.50	15.00	6750	1.481
Computer	g	13×10^3	0.33	15.00	6750	0.642
Plastic glasses	g	2.27	5.00	1	1	11.350
Deionised water	g	270	1.00	1	1	270.000
NaOH (0.1 M) as titrant	g	17.5	1.00	1	1	17.500
Buffer solution (pH 4)	g	50	2.00	1	1	100.000
Buffer solution (pH 7)	g	50	2.00	1	1	100.000
Manual pipettes (1–10 mL)	g	114.81	1.00	10	4500	0.026
Pipettes caps (1–10 mL)	g	5.85	0.10	1	1	0.585
Water (for cap washing)	g	5	1.00	1	1	5.000
Electricity	kWh	0.059	1.00	n.a.	1	0.059
Output						
Plastic waste	g	17.1	n.a.	n.a.	n.a.	n.a.
Chemical waste	g	240	n.a.	n.a.	n.a.	n.a.

2.2.4. Determination of pH LCI. Similar to the TA, pH was determined with an automatic potentiometric titrator. In this case, 50 mL of must sample and two buffer solutions were used. The titrator performed the analysis automatically, and no more chemicals or reagents were used. Water was accounted for routine procedure of washing analytical tools and workspace. The input and output concerning this chemical analysis are illustrated in Table 3.

Table 3. Quantity, allocation factor, lifetime and amount per analysis for pH determination.

	Unit	Quantity (Q)	Allocation factor (Af)	Lifetime (years)	Lifetime (No. analyses)	Amount per analysis
Input						
Must	g	50	1.00	n.a.	1	50.000
Automatic volumetric titrator	g	20×10^3	0.50	15.00	6750	1.481

Computer	g	13 x 10^3	0.33	15.00	6750	0.636
Plastic glasses	g	2.27	3.00	1	1	6.810
Deionised water (for workspace washing)	g	200	0.10	n.a.	1	20.000
Deionised water (for instrument washing)	g	200	0.20	n.a.	1	40.000
Buffer solution (pH 4)	g	50	2.00	1	1	100.000
Buffer solution (pH 7)	g	50	2.00	1	1	100.000
Electricity	kWh	0.059	1.00	n.a.	1	0.059
Output						
Plastic waste	g	6.81	n.a.	n.a.	n.a.	n.a.
Chemical waste	g	210	n.a.	n.a.	n.a.	n.a.

2.2.5. Determination of TSS LCI. Must TSS was determined with a digital refractometer: for each sample analyzed, about 100 µL of must was placed within the analytical tool and the TSS measured. This procedure can also be carried out in field without extra sample preparation. Considering the aim of the study, the worst scenario was analysed. The input and the output related to the determination of TSS are reported in Table 4.

Table 4. Quantity, allocation factor, lifetime and amount per analysis for TSS determination.

	Unit	Quantity (Q)	Allocation factor (Af)	Lifetime (years)	Lifetime (No. analyses)	Amount per analysis
Input						
Must	g	0.2	1.00	n.a.	1	0.200
Digital refractometer	g	200	1.00	15	6750	0.030
Manual pipettes (20–200 µL)	g	80.6	1.00	10	4500	0.018
Pipettes caps (20–200 µL)	g	0.38	1.00	1	1	0.380
Paper towel (for instrument cleansing)	g	2.03	0.33	n.a.	1	0.677
Water (for instrument washing)	g	0.015	1.00	n.a.	1	0.015
Output						
Plastic waste	g	0.38	n.a.	n.a.	n.a.	n.a.
Paper waste	g	0.892	n.a.	n.a.	n.a.	n.a.

2.2.6. Benchtop VIS/NIR spectroscopy LCI. The use of visible and near-infrared spectroscopy (VIS/NIR) can provide an objective, repeatable, rapid, accurate, and nondestructive method for the evaluation of the composition of grapes. Without any sample preparation, the undamaged grape samples were analyzed using the light from the VIS/NIR spectrophotometer.

The basic concept of VIS/NIR spectroscopy is that the light coming from the instrument is reflected by the grapes and the spectra obtained is then analyzed and visualized by a software for model calibration within a computer. From the spectral result, a wide range of information can be deduced after only one single analysis.

The only devices necessary for this type of technology are the VIS/NIR spectrophotometer and the computer; in addition to that, no chemicals are required, and the samples can be analyzed directly in the field without producing any waste; thus, no outputs were considered in the VIS/NIR inventory. All the input concerning the optical analysis are reported in Table 5.

A fundamental matter concerning VIS/NIR technology is the calibration phase that allows reliable results to be obtained, although they are not a direct measurement of the quality parameters, but only their estimation. The calibration is designed to be a process that entails 700 conventional analyses per parameter, which correspond to 700 optical analyses conducted on the same sample. Five hundred calibration analyses were performed at the beginning of the optical method, with the other 200 analyses used as a validation test. In this study, three models for TA, pH and TSS were allocated to the 6750 analyses undertaken in 15 years.

Table 5. Quantity, allocation factor, lifetime and amount per analysis for VIS/NIR spectroscopy analysis.

	Unit	Quantity (Q)	Allocation factor (Af)	Lifetime (years)	Lifetime (No. analyses)	Amount per analysis
Input						
VIS-NIR spectrophotometer	g	15×10^3	1.00	15	6750	2.22
Computer	g	13×10^3	1.00	15	6750	1.926
Calibration	*n*	2100	0.015	15	6750	0.004666667
Electricity	kWh	0.0564	1.00	n.a.	1	0.0564

2.2.7. Simplified and portable VIS/NIR Prototype LCI. Another type of optical method used a simplified and portable LED-based prototype. This prototype device (technology readiness level equal to 5) is composed by tuned photodiode arrays, interference filters, LEDs and optics. In detail, the device incorporates two digital sensors (ams-OSRAM, models AS7262 visible and AS7263 NIR, Premstätten, Austria) for spectral measurement in the visible (VIS) and Short Wave Near-infrared (SW-NIR) region. The VIS and SW-NIR sensors are 4.5 × 4.4 mm in size and are classified as ultra-low power consumption sensors. They have 12 independent on-device optical filters to detect reflectance light at 450, 500, 550, 570, 600, 610, 650, 680, 730, 760, 810 and 860 nm [17]. Optical data were acquired on both bunches and single berries directly in the field without any sample preparation. Data were collected using the LED fully integrated prototype to avoid sample degradation. The input referring to this optical analysis are collected in Table 6. Also, for this analysis with the portable device, a calibration procedure was based upon the benchtop device calibration model.

Table 6. Quantity, allocation factor, lifetime and amount per analyses for portable device.

Input	Unit	Quantity (Q)	Allocation factor (Af)	Lifetime (years)	Lifetime (No. analyses)	Amount per analysis
Prototype	g	130	1.00	15	6750	0.019
Calibration	*n*	2100	0.015	15	6750	0.004666
Electricity	kWh	0,000093	1.00	n.a.	1	0.000093

2.2.8. Performance Characterisation Factor. As described above, the two optical instruments (benchtop and prototype) do not provide a direct measurement of the compositional parameters, only their estimation; chemical procedures, however, do provide a direct measurement. Thus, the prediction capacity of the instruments was considered an important aspect that contributes to the final environmental impact interpretation. To obtain the performance values of the two instruments, two common main factors were considered: the mean and the root mean square error of cross-validation (RMSEcv). Both instruments were used on the same grapes; regarding the benchtop VIS/NIR device, the values of the mean and the RMSEcv were taken according to Casiraghi et al. [23]; while the same two factors referred to the prototype were collected from Pampuri et al. [17].

2.2.9. Life cycle impact assessment (LCIA). The impact assessment phase of LCA aimed at evaluating the significance of potential environmental impacts using the results of the life cycle inventory analysis. In general, this process involves associating inventory data with specific environmental impacts and attempting to understand those impacts. The level of detail, choice of impacts evaluated, and methodologies used depend on the goal and scope of the study [19].

To analyse the environmental impact, the SimaPro v 9.1.1.1. (PRé Sustainability, Amersfoort, The Netherlands) software was used. ReCiPe 2016 midpoint (H) was used as the method to calculate the environmental impact related to the chemical and the optical analyses for the measurement of the three quality parameters of grapes. ReCiPe 2016 considers 18 impact categories which are reported in Table 7.

Table 7. Impact categories, acronyms and unit of ReCiPe 2016 midpoint (H) method.

Impact category	Acronyms	Unit
Global warming	GWP	kg CO_2 eq
Stratosheric ozone depletion	ODP	kg CFC11 eq
Ionising radiation	IRP	kBq Co-60 eq
Ozone formation - human health	HOFP	kg Nox eq
Fine particular matter formation	PMPF	kg PM2,5 eq
Ozone formation - terrestrial ecosystems	EOFP	kg Nox eq
Terrestrial acidification	TAP	kg SO_2 eq
Freshwater eutrophication	FEP	kg P eq
Marine eutrophication	MEP	kg N eq
Terrestrial ecotoxicity	TETP	kg 1.4-DCB
Freshwater ecotoxicity	FET	kg 1.4-DCB
Marine ecotoxicity	METP	kg 1.4-DCB
Human carcinogenic toxicity	HTPc	kg 1.4-DCB
Human non carcinogenic toxicity	HTPnc	kg 1.4-DCB
Land use	LU	m^2a crop eq
Mineral resource scarcity	SOP	kg Cu eq
Fossil resource scarcity	FFP	kg oil eq
Water consumption	WCP	m^3

3. Results and Discussion

According to the purpose of the study, the chemical analysis, the optical analysis with benchtop instrument spectroscopy and with the prototype were first analysed separately and then compared with each other. The results included the hotspot identification criterion for the single study of the three approaches. For the comparison, a numerical and quantitative concept was needed including all the three possible solutions together (chemical analysis, optical analysis with benchtop spectrometer and optical analysis with portable device).

3.1. Conventional methods LCIA. Table 8 represents the environmental impact in percentages, highlighting the main hotspots deriving from the inputs and outputs of the pool of chemical analyses. To better interpret the results concerning this method, the final environmental impact was divided into the three chemical analyses that allow the three quality parameters of grapes to be obtained. Among these three chemical analyses, the ones which appear to have the highest environmental impact are the determination of pH and TA, which has an impact range of 40–50% in almost every impact category.

This result is explained by using a certain quantity (100 g) of buffer solution that is fundamental for the calibration of the automatic potentiometric titrator, used for TA and pH analyses. Only three impact categories (MEP, LU and WCP) see the pH determination as mainly responsible for the environmental impact reaching values of 79.1, 82.2 and 76.5%, respectively, and therefore define a lower level of environmental responsibility for the determination of TA. For TSS, the environmental responsibility reaches a maximum of 1% in all the impact categories, thus not appearing environmentally significant, due to the use of a simplified system such as the refractometer for its determination.

Table 8 underlines the most environmentally impactful analysis, although it is unable to convey the reason. Thus, according to the goal of the study, the three wet-chem analyses must be treated as a single chemical analysis represented by a pool. To interpret the results deriving from the pool, its inputs and outputs were subdivided into seven factors (analytical tools, chemicals, energy, waste, laboratory materials, sample and calibration) allowing the identification of the main hotspots.

Table 8. Environmental impact percentage responsibilities of the three chemical analyses.

Impact Category	Unit	Total	Percentage responsibilities (%)		
		Pool of three chemical analysis	Total acidity	pH	Sugars
GWP	kg CO_2 eq	1.66	50.7	49.0	0.3
ODP	kg CFC11 eq	2.40 x 10^{-6}	45.1	54.8	0.1
IRP	kBq Co-60 eq	6.69 x 10^{-2}	58.8	40.4	0.9
HOFP	kg NOx eq	2.16 x 10^{-3}	47.0	52.5	0.5
PMPF	kg $PM_{2.5}$ eq	1.45 x 10^{-3}	47.5	51.9	0.6
EOFP	kg NOx eq	2.26 x 10^{-3}	47.0	52.5	0.5
TAP	kg SO_2 eq	3.72 x 10^{-3}	45.9	53.5	0.6
FEP	kg P eq	4.45 x10^{-4}	49.1	50.4	0.5
MEP	kg N eq	2.89 x10^{-4}	20.4	79.1	0.5
TETP	kg 1.4-DCB	3.39	49.0	50.6	0.4
FET	kg 1.4-DCB	1.25 x 10^{-1}	39.9	59.7	0.4
METP	kg 1.4-DCB	1.23 x 10^{-1}	48.5	51.1	0.4
HTPc	kg 1.4-DCB	4.61 x 10^{-2}	47.4	52.2	0.4
HTPnc	kg 1.4-DCB	1.84	42.4	57.0	0.5
LU	m^2a crop eq	1.76 x 10^{-1}	16.6	82.2	1.2
SOP	kg Cu eq	1.39 x 10^{-2}	48.3	51.6	0.2
FFP	kg oil eq	3.15 x 10^{-1}	50.6	49.0	0.5
WCP	m^3	4.71 x 10^{-2}	23.0	76.6	0.4

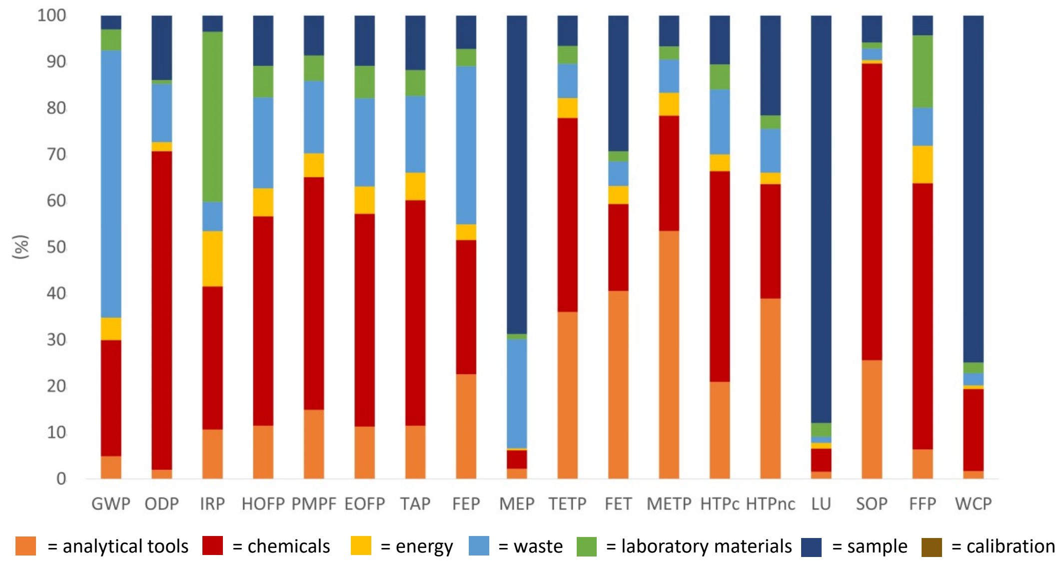

Figure 2. Hotspots for the pool of chemical analyses based on a factor subdivision. Impact categories and acronyms are defined in Table 7.

As shown in Figure 2, the use of chemicals is the most impactful factor among those cited above in almost all the impact categories (9 out of 18) with values ranging from 45% to 70%. This factor refers to all the solutions and reagents used during chemical procedures, such as deionized water, sodium hydroxide and all the buffer solutions used to calibrate the automatic potentiometer. Only for WCP, LU and MEP, the main responsible for the environmental impact is the preparation of the sample: for the marine eutrophication, the sample impacts for a 69%, due to the use of chemicals during the cultivation phase of the grapes that are washed away causing a trophic enrichment of the water. Regarding the land use impact category, the sample has a percentage value of responsibility of 88% due to the occupation of the field for the growth of the grapes; instead for what concerns the water consumption, an impact of 75% can be observed justified by the high consumption of water for the irrigation of the vineyard.

3.2. Benchtop vis/NIR and prototype optical analyses LCIA. The VIS/NIR approach does not need the same inputs and outputs as the conventional method, thus only three factors needed to be identified, according to the subdivision criteria: analytical tools, energy and calibration. Figure 3 represents the main responsible hotspots of the environmental impact regarding the benchtop technology and the one with the prototype. The hotspot with the highest environmental responsibility in all the impact categories considered is the calibration phase which requires a high number of chemical analyses to calibrate the predictive model to define the quality parameters for the grapes. The energy seems to be the second hotspot for the benchtop solution, due to its low consumption during the optical procedure.

As observed in Table 8 for the determination of TSS, the use of simplified systems permits a significant reduction of the environmental impacts. Therefore, this study included also this type of system for the determination of the three quality parameters simultaneously and not only for one single parameter as in the conventional analysis. Therefore, regarding the optical methods, not only the benchtop device was considered, but also a portable prototype. In Figure 3, the environmental impact of this second type of optical method is also reported highlighting the main responsible hotspots for the procedure, the subdivision follows the same as the benchtop device (analytical tools, energy and calibration). The calibration factor is the principal hotspot also for this kind of optical system in all the impact categories with a percentage responsibility that in some impact categories reaches values of 100 %. The analytical tools instead have an impact percentage so small (less than 5%) that they were considered as a negligible factor.

To compare the environmental impact of the conventional analysis, the optical analysis with benchtop VIS/NIR and the one with the prototype, a quantitative evaluation is needed. Table 9 reports the comparison of the three methods showing their environmental responsibility, in addition to the ratio values for each impact category which consider the pool of three chemical analyses compared with one optical analysis with the benchtop solution and one optical analysis with the portable solution.

Results show that the chemicals analyses have the highest environmental impact in all the impact categories, instead of the optical analyses with the benchtop instrument and the prototype, the environmental impacts are similar, even though the benchtop VIS/NIR solution seems to have a higher impact due to the necessity of having a computer to analyze the spectra obtained by the instrument, the portable device, on the other hand, does not require a PC because it's used directly on the field and as shown in Figure 3 (the analytical tools are not significant in terms of environmental impact).

In addition to the environmental impact, the ratio % results highlight the differences between the two optical analyses even though there is not a wide variability: it can be observed that an average ratio of 37% is related to the benchtop solution concerning the environmental impact of the pool of the three chemical analyses, while for the prototype an average value of 31% is considered. These results identify once again the advantages of using the prototype solution which allows different parameters to be obtained with one single analysis thanks to a simplified system.

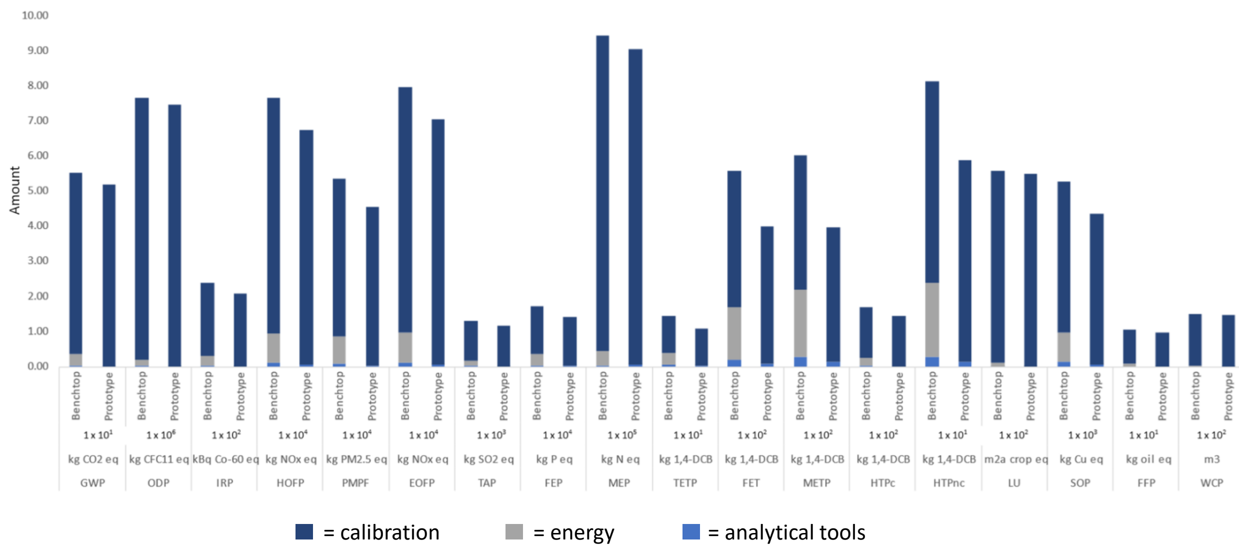

Figure 3. Hotspots for the benchtop VIS/NIR and the prototype devices based on the reference parameters. Impact categories and acronyms are reported in Table 7.

Table 9. Impact values related to the execution of the three methods (chemical optical with benchtop device and optical with portable device) to measure the three reference parameters to characterise grapes.

Impact category	Unit	Pull of three chemical analyses	Ratio % benchtop	Ratio % prototype
GWP	kg CO_2 eq	1.66	33	31
ODP	kg CFC11 eq	2.40×10^{-6}	32	31
IRP	kBq Co-60 eq	6.69×10^{-2}	36	31
HOFP	kg Nox eq	2.16×10^{-3}	35	31
PMPF	kg $PM_{2,5}$ eq	1.45×10^{-3}	37	31
EOFP	kg Nox eq	2.26×10^{-3}	35	31
TAP	kg SO_2 eq	3.72×10^{-3}	35	31
FEP	kg P eq	4.45×10^{-4}	39	32
MEP	kg N eq	2.89×10^{-4}	33	31
TETP	kg 1.4-DCB	3.39	43	32
FET	kg 1.4-DCB	1.25×10^{-1}	45	32
METP	kg 1.4-DCB	1.23×10^{-1}	49	32
HTPc	kg 1.4-DCB	4.61×10^{-2}	37	31

HTPnc	kg 1.4-DCB	1.84	44	32
LU	m^2a crop eq	1.76 x 10^{-1}	32	31
SOP	kg Cu eq	1.39 x 10^{-2}	38	31
FFP	kg oil eq	3.15 x 10^{-1}	34	31
WCP	m^3	4.71 x 10^{-2}	32	31

The results reported in Table 9 underline the importance of having a simplified device as it is the greenest solution among those evaluated in this study, allowing a general reduction of the environmental impact in all the impact categories. Nevertheless, this statement cannot be assumed as completely reliable as the higher criticism is linked to the necessity to consider also the performance of the instrument which can be identified as a limiting factor. To evaluate the change of impact deriving from taking into consideration the performance of the optical devices, a normalisation of the results concerning this limiting factor was performed.

3.3. Performance Adjusted/Based Functional Unit. Considering environmental aspects, the prototype appears to be the best choice in determining the three compositional parameters for the characterisation of the grapes, but its performance may be not so reliable in obtaining precise and trustworthy results. For this reason, as cited in the inventory phase for the optical analyses, also the performance factor was included. Therefore, the study compared the two optical methods (benchtop and prototype) concerning the conventional analyses in order to observe how results change if focusing the attention not only on the environmental impact but also on the performance of the devices.

Table 10 represents the performances of the three scenarios analysed. Three main factors were taken into consideration: the mean, the root mean square error of cross-validation (RMSEcv) and the final performance of the procedures. The three chemical methods were considered as the best choice in terms of reliability of results assuming an error equal to zero and a performance that reaches a value of 100%. Assuming that the three chemical analyses are the best solution to obtain results without errors, between the two optical methods, the benchtop device appears to have a better performance in determining all the three quality parameters (97% for TSS, 89% for TA and 98% for pH) with respect to the prototype device that seems to be less precise in terms of values. The last column of Table 10 reports also the correction factor (CF) which was calculated by relating the average performance (Ap) of the chemical analyses (equal to 100%) with the average performance (Ap) of the benchtop and prototype respectively. For the benchtop, a correction factor of +6% must be applied to reach the same performance as the chemical analyses, while for the prototype solution the CF is equal to +7%. These results highlight how the benchtop solution is an effective choice which produces similar and precise values to those obtained with the conventional method compared to the prototype, even though the LCA study recognises it as the better solution in terms of environmental impact.

According to the normalisation of the results concerning the performance of the two optical instruments, the gap between the pool of the three chemical analyses and the two optical solutions decreases. Despite this change in the gap and even if the benchtop solution has higher performance with percentage points between +1 and +3% compared to the performance of the simplified system, the prototype is once again the most convenient solution compared to the benchtop one because it needs a lower number of analyses to reach the same results as those obtained from the pool of the three chemical analyses.

The results of the environmental impacts of the two optical analyses compared to the pool of the three chemical analyses after the performance evaluation are reported in Figure 4 which recognises the prototype device as the best solution in terms of environmental impact compared to the benchtop instrument.

Table 10. Mean, root mean square error of cross validation, performance, and correction factor of the three methods under study; ***m****= mean,* ***$RMSE_{cv}$****= root mean square error of cross validation;* ***p****= performance;* ***Ap****=average performance.* ***CF****= correction factor.*

	Sugars			**Total acidity**			**pH**			**3 analyses**	**CF**
	m	**$RMSE_{cv}$**	**p**	**m**	**$RMSE_{cv}$**	**p**	**m**	**$RMSE_{cv}$**	**p**	**Ap**	
Benchtop	21.45	0.7	97%	10.22	1.09	89%	3.215	0.08	98%	95%	**1.06**
Prototype	22.9	1.31	94%	6.9	0.83	88%	3.33	0.09	97%	93%	**1.07**

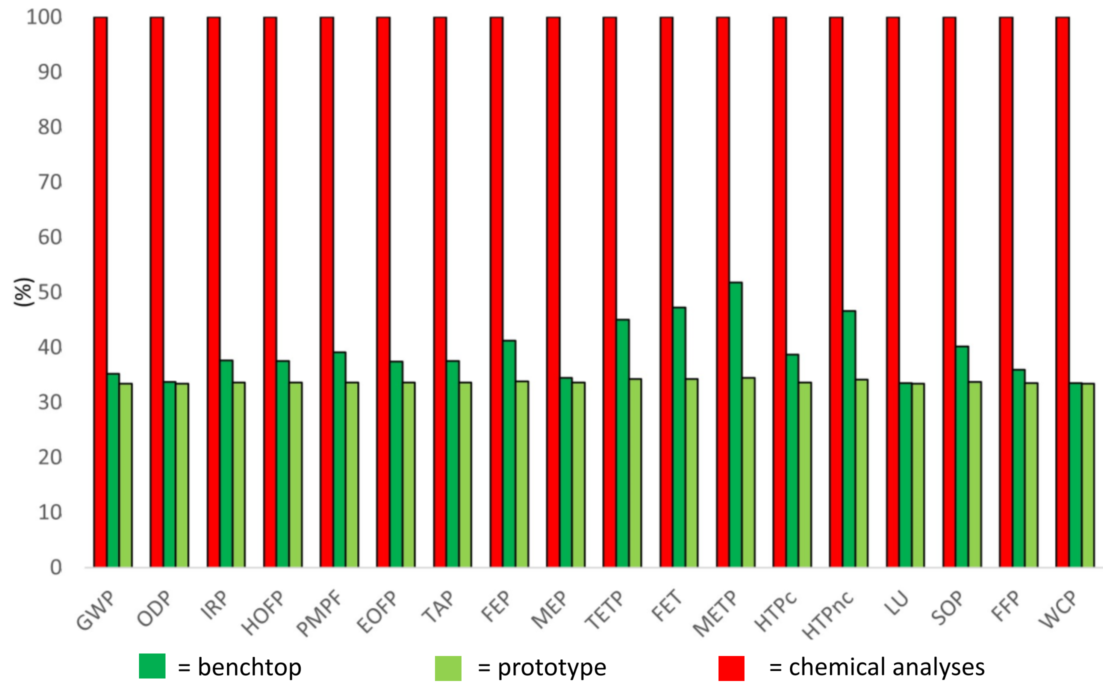

Figure 4. Comparison between the environmental impact of the two optical analyses, benchtop and prototype, compared to the pool of the three chemical analyses after the performance evaluation.

4. Conclusions

The optical analyses are already validated systems in food production to reduce the environmental impact along the chain by adopting not only benchtop solutions but also portable and simplified technologies. The environmental impact of wet-chem analyses, optical with benchtop and optical with the simplified prototype were analysed and compared in this study to characterise and evaluate the quality of grapes. The results obtained from the study highlight that the use of chemicals during the procedures of the wet-chem analyses is the main driver of the environmental impact, while for what concerns the optical analyses with the two different instruments, the calibration phase is the most impactful factor. At the end of the study, it was possible to define the optical analysis with the prototype as the most suitable and greenest solution to obtain the three quality parameters. Nevertheless, the study did not consider the variability of results due to the performance of the two optical devices, therefore an additional observation was made normalising the results obtained with respect to a performance factor. Considering only the performance, the benchtop solution is the best choice because it produces similar and precise values to those obtained with the conventional method, even though it has a higher environmental impact compared to the prototype device. After this evaluation, results were normalised to the performance of the two devices and the results showed how the optical analysis with the portable device is once again the best solution to obtain much more reliable measurements compared to the benchtop instrument. Research should consider the environmental advantages of these optical analyses relating them to a performance evaluation to identify the best solution in terms of both performance and environmental impact. This study could be a starting point for further works which will consider a similar environmental comparison but applying it to different agri-food products that need other kinds of quality analyses. Innovations in agriculture and the development of smart solutions could represent advantages of managing and monitoring agri-food product quality and propose actions to reduce waste and the impact of the agri-food supply chain in a view of agriculture 4.0.

Acknowledgments

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4.3 SPECTROSCOPY FOR REAL-TIME QUALITY CONTROL IN THE WINERY

As discussed in previous chapters, the use of sensors and enabling technologies is becoming increasingly prevalent in the wine industry. Visible and near-infrared (vis/NIR) spectroscopy has been applied at various stages of the production chain, from grape analysis to fermentation monitoring and even wine analysis, for the identification of color and aromatic profiles. The reliability of this technique in creating predictive models to monitor quality parameters such as sugar content, acidity, or pH has already been demonstrated. However, one of the unique features of spectroscopy is its ability to obtain information on multiple parameters through a single analysis. The objective of this experiment was, therefore, to verify the applicability of this technique directly in the winery, enabling the measurement of multiple grape parameters with a single analysis. This approach aims to provide significant support to the industry by ensuring the quality of the raw materials and guaranteeing a final product of the highest quality.

PAPER 4: Grape polyphenol content prediction through vis/NIR spectroscopy in a view of real time application at winery consignment

Alessia Pampuri, Alessio Tugnolo, Valentina Giovenzana, Sara Vignati, Roberto Beghi and Riccardo Guidetti

Abstract

The evaluation of grapes at the consignment stage is critical for producing a high-quality product, especially for large companies such as cooperatives, where there are many members from different vineyards and differing growing and cultivation methods. These factors can result in the production of grapes of the same variety with different quality status. It is therefore essential to develop methods for objectively and accurately evaluating the grapes quality to optimize the initial selection phase for various winemaking processes. The aim of the study was to explore the feasibility of vis/NIR spectroscopy for rapidly assessing the polyphenol content in grapes, initially on a laboratory scale and subsequently transitioning to practical implementation in the winery.

To conduct the experiment, with a distance of 150 mm between the sensor and the sample, a process spectrometer (with a spectral range of 380-1650 nm) was used. Data on bunches of Vitis vinifera L. (Ancellotta variety) were collected using a setup that was reproducible in the winery to lay the foundations for further experimentations. Partial least squares (PLS) regression method was used to predict polyphenol content using the acquired spectra. Model results showed that the system was able to provide valuable information on phenolic parameters. The model for predicting total polyphenols reported an RMSE of 227 mg/L and 315 mg/L in calibration and cross-validation,

respectively, along with an R^2 of 0.86 and 0.73 in calibration and cross-validation, respectively. The use of this technology certainly has the potential to improve the management of winemaking processes by providing reliable, sustainable and rapid results and also providing the possibility of carrying out quality controls on the matrix at different points of the supply chain.

Introduction

The evaluation of grapes during the consignment phase is a vital step towards producing a high-quality final product. This is especially true for large companies like cooperatives, where the number of members is significant, and vineyards possess different soils, growing techniques, and cultivation methods. These factors can lead to the production of grapes of the same variety with varying qualitative characteristics. To ensure the production of high-quality grapes, it is important to develop methods for objective and accurate evaluation of their quality (dos Santos Costa et al., 2019). This can optimize the initial selection phase for various winemaking processes and ultimately result in a better final product.

Given its high added value, the quality of wine certainly depends on numerous factors, including the composition of the starting grapes and their content of substances such as polyphenols. Polyphenols are a group of compounds that significantly contribute to the sensory characteristics of wine, including color, flavor, aroma, and astringency. Therefore, the quality of the grapes at the time of harvesting is crucial for producing high-quality wine (Gutiérrez-Escobar et al., 2021).

Traditionally, grape quality has been evaluated using physical and chemical analysis, which can be time-consuming, expensive, and destructive to the grapes. In recent years, Near Infrared (NIR) and Visible (vis) spectroscopy has emerged as a potential tool for rapid, non-destructive and cost-effective assessment of grape quality. The technology is based on the measurement of the interaction of light with the chemical components of grapes, including polyphenols (Rouxinol et al., 2022).

Visible/Near-Infrared (vis/NIR) spectroscopy has been shown to be useful for monitoring and determining various quality parameters of grapes at different points along the wine production chain. This technique can be applied to assess sugar content, pH, acidity, color parameters, and many others (Ye et al., 2022) that is, capable of determining both technological and phenolic maturity of the grapes. It is a non-destructive optical technique that do not involve sample destruction and can be performed on solid samples such as grape berries or bunches, as well as on liquid or semi-liquid samples such as musts or even wine.

In addition to being a non-destructive technique, with consequent reduction of waste regarding production controls, it is also rapid, user-friendly, and does not require a particular sample preparation. Therefore, it is overall considered a promising green and sustainable technique capable of providing useful information in a short time to monitor multiple aspects (Grassi et al., 2021).

Throughout the entire grape-wine production chain, various quality controls can be carried out, ranging from monitoring vine health, managing irrigation, to direct controls on grapes to plan the most suitable time to start harvesting, to all the controls carried out after harvesting the bunches. In particular, with the development of portable optical instruments that use vis/NIR technology, many of these controls can be carried out in real time by operators for qualitative analysis of parameters and for the determination of some fruit metabolites (Power et al., 2019). Facilitating the phases of grape control and inspection when it arrives in the winery using spectroscopy would indeed constitute a significant advancement towards viticulture 4.0, as it is a crucial phase in ensuring the quality of the finished product.

Using an objective technique like vis/NIR spectroscopy to assess grape quality at consignment could improve quality standards. This is particularly relevant in productions where operator experience is still often used as a measure. Additionally, it would provide guarantees to wine producers. These producers must pay suppliers from external markets according to prices defined by contracts or interprofessional agreements (Pomarici et al. 2021).

The aim of this experimentation was to test an experimental setup on a laboratory scale. This setup could later be reproduced directly in the winery. It was designed to acquire spectroscopic optical data from mechanically harvested grapes. These data were then correlated with destructive analyses, used as reference parameters, to create predictive regression models.

A growing number of grape growers are keen on implementing mechanization in their vineyards. Therefore, it is crucial to gain a deeper understanding of how mechanization impacts the quality of berries and wine. The field of vineyard mechanization is advancing swiftly, with numerous studies examining its effects on various practices such as harvesting, pruning and leaf removal (Sun et al., 2022).

For the experimentation, Ancellotta grape variety was used: a dark-skinned grape with high coloring power, mainly cultivated in northwestern Italy, Brazil, the southeast of Switzerland, and Argentina (Alvarez Gaona et al., 2019). From this variety, red wines with a high polyphenolic content are produced (Gaona et al., 2022).

Materials and methods

Sampling

The experimentation involved samples of Ancellotta grapevines (*Vitis vinifera* L.) over two consecutive growing seasons. The experimental campaign of the first year was conducted by collecting Ancellotta grape intact bunches. The samples were collected by the producers between August 17 and September 14, 2021, the day of delivery at the winery by all contributors. In total, for the first year of experimentation, 90 samples were collected, divided into 10 sampling days. To conduct the feasibility study, based on the winery's requirements, the grape bunches were thawed and partially crushed to closely simulate mechanical harvesting. The samples were optically analyzed at the laboratories of the Department of Agricultural and Environmental Sciences of the University of Milan.

After the quite satisfactory results of the first year, particularly regarding the predictive models for anthocyanins and total polyphenols, 68 real samples of the same grape variety from mechanical harvesting were analyzed during the second year of experimentation. Real samples were analyzed at the time of consignment to scale up the experiments; the second trial was, in fact, conducted directly in the winery using the same instrument and the same acquisition conditions as in the first year. Additionally, 100 samples of whole grape bunches at different stages of ripeness were analyzed, as was done during the first experimentation campaign.

Reference analysis

The destructive analyses of the first year of experimentation were conducted by an external laboratory. In accordance with the winery's requirements, certain parameters were selected for the reference destructive analyses in the second year of sampling: total soluble solids, anthocyanins at pH 1, total anthocyanins and total polyphenols. Also in this case, the whet chemical analyzes were commissioned to an external laboratory.

Instrumentation Features and Optical Acquisitions Setup

Optical acquisitions both in the university laboratory and the winery (in the second year of experimentation) were performed using the same instrument: a spectrophotometer that operates in the spectral range between 380 and 1650 nm, covering the visible and Near-Infrared portions of the electromagnetic spectrum.

Figure 1. Corona® Process spectrophotometer.

The instrument used is the Corona® Process (Carl Zeiss, Oberkochen, Germany), a spectrophotometer that can be used both as a bench instrument and as a process instrument as it can be mounted on conveyor belts or movable arms. Optical acquisitions were acquired with the samples positioned 15 cm away from the sensor as shown in Figure 4, allowing for consistent analysis both in the laboratory (controlled conditions) and in the winery thanks to the instrument's characteristics.

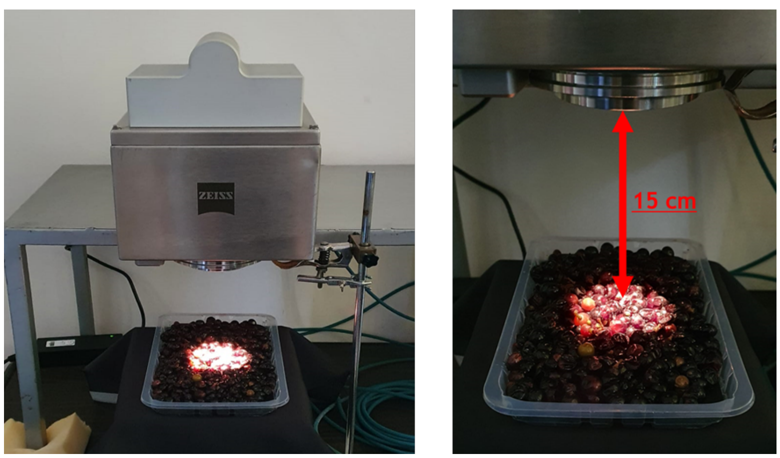

Figure 2. Spectral acquisition setup carried out on the grape samples.

Three acquisitions were collected for each sample, subsequently averaged to obtain a single spectrum representative of the sample.

<u>Data Processing and Development of Regression Models</u>

Chemometrics analysis was performed in Matlab environment, version 2023b (MathWorks, Inc., Natick, MA, USA) using the PLSToolbox package (Eigenvector Research, Inc., Manson, WA, USA). To correlate the reference destructive analyzes with the optical acquisitions, in the chemometric approach it is essential to follow the different steps to analyze the data correctly to extract the most useful information. The first step was the creation of data matrices and then the execution of an unsupervised exploratory analysis: Principal Component Analysis (PCA). The primary purpose of Principal Component Analysis (PCA) is to reduce the dimensionality of the original dataset by identifying and explaining, where present, the correlations among a large number of variables and a smaller number of principal components (PCs) without losing significant information. By utilizing PCs, it is possible to examine the relationships between samples and visualize trends (Iorgulescu et al., 2016). With PCA is also possible to apply an outlier detection procedure using the "Hotelling T2 computation" function.

Following the unsupervised exploratory analysis, regression was performed with the aim of modeling one or more response variables (Y, in this case, the qualitative parameters of interest for the winery) using a dataset of predictors (X, in this case, consisting of spectra) (Wold et al., 2001).

The reference parameters were used for the calculation of Partial Least Square predictive models. PLS models are widely employed in the context of spectral analysis (Lavine and Workman, 2013; Ferrara et al., 2022) because they integrate the principles of PCA and linear regression, consolidating predictors into latent variables (LVs). The latent variables are therefore linear combinations of predictors, are orthogonal to each other, and are constructed based on the information contained in the dependent variables. To evaluate model accuracy Root Mean Square Error (RMSE), bias and coefficient of determination (R^2) were used. RMSE and bias have to be as low as possible, while R^2 has to be as high as possible (with a maximum equal to 1) to prove a good model performance.

Results and discussion

In Figure 3 are reported the box plots with the results in terms of pH, total soluble solids (TSS) content measured in Brix degrees, total anthocyanin content expressed in mg/L of malvidin chloride, and total polyphenol content expressed in mg/L of gallic acid.

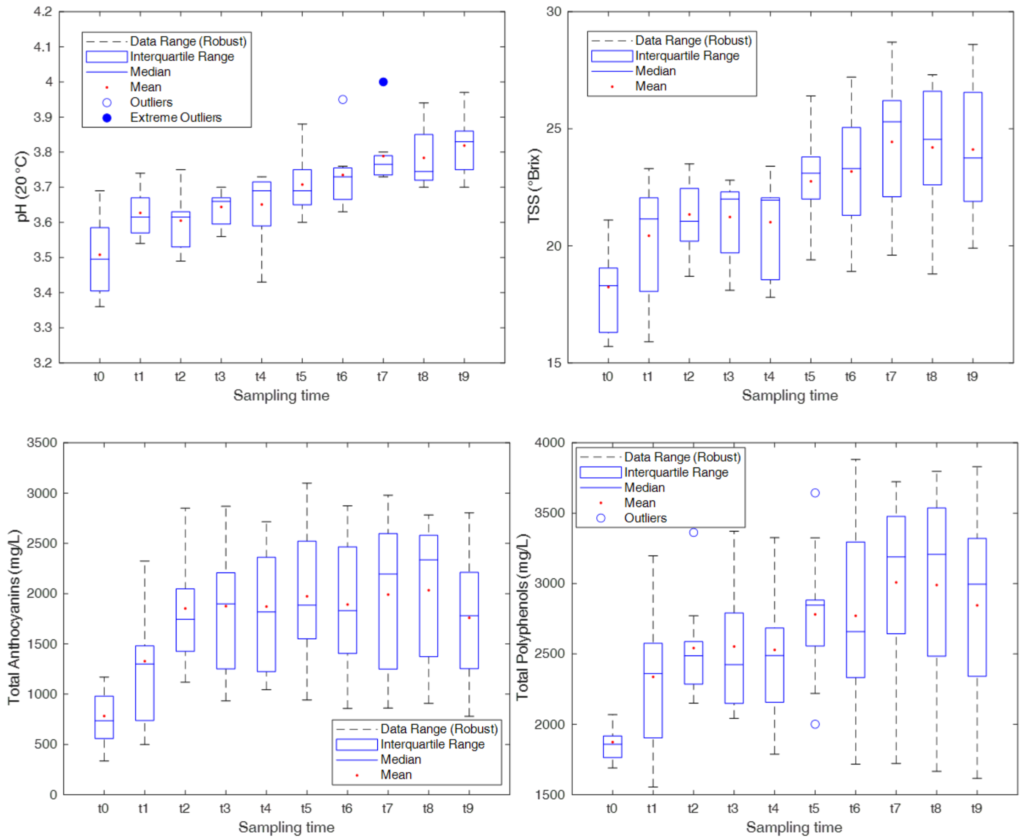

Figure 3. Boxplot representing destructive analysis of the first year of sampling.

It is possible to observe the characteristic curves of these parameters as maturation progresses: pH, sugar content, as well as anthocyanin and polyphenol content tend to increase as maturation proceeds (Zhao et al., 2019). These are oenological parameters that are not always uniform or regular within the same vineyard, often due to different viticultural practices or different soils or climatic conditions (Matese & Di Gennaro, 2015). Precisely for these reasons, they are parameters to be kept under control and continuously monitored, causing a waste of money and time that is leading towards the attempt to use portable and rapid measurement tools (Schorn-Garcia et al., 2023). Given the importance of these parameters, among the available destructive analyses of the first year of the experimental campaign, these four parameters were selected and used for the creation of predictive models.

Figure 4 presents the PLS models for predicting soluble solids content and pH. The model for TSS (Figure 6a) shows a Root Mean Square Error (RMSE) of 0.74 in calibration and 1.30 in cross-validation, with an R^2 of 0.93 and 0.80, respectively. The model for pH (Figure 6b) is less efficient, with an RMSE of 0.04 in calibration and 0.08 in cross-validation, and a coefficient of determination of 0.86 in calibration and 0.53 in cross-validation.

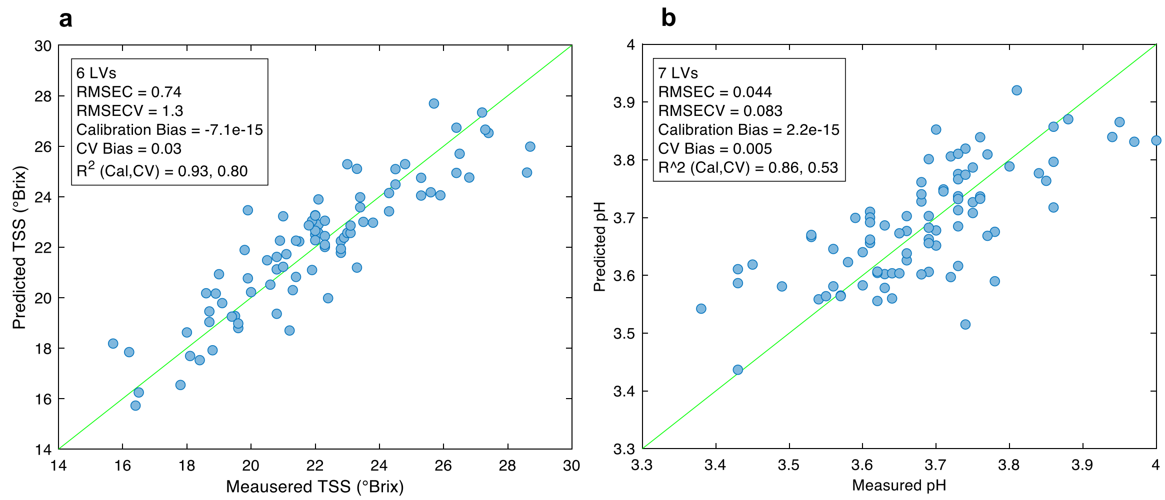

Figure 4. Predictive models for TSS (a) and pH (b).

Figure 5 shows the two predictive models for total anthocyanins (a) and total polyphenols (b). The model for anthocyanin prediction has a slightly higher R² compared to the model for total polyphenols (0.89 and 0.76, respectively, versus 0.86 and 0.73 for the latter), though it also exhibits a higher error. Nonetheless, these are solid models with promising initial results that warrant further investigation through additional experiments, testing a larger number of real samples, and implementing the instrument to perform true real-time analyses.

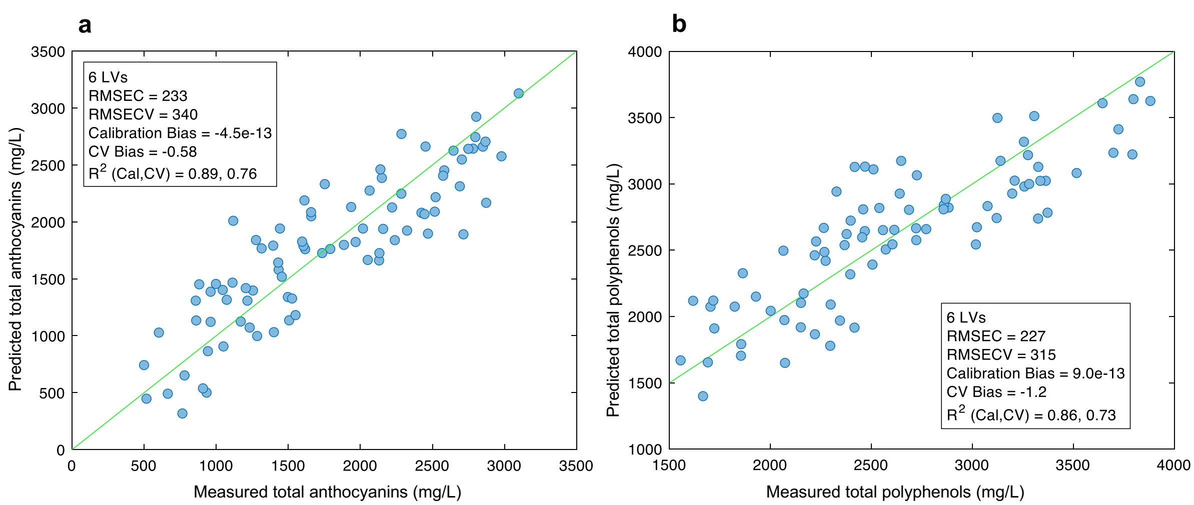

Figure 5. Predictive models for total anthocyanins (a) and total polyphenols (b).

The results from the first year of experimentation, in fact, laid the groundwork for the second year of trials. The findings were promising, supporting the decision to conduct the second experimentation using the same instrument and acquisition setup, but with real samples collected at the time of consignment to the winery.

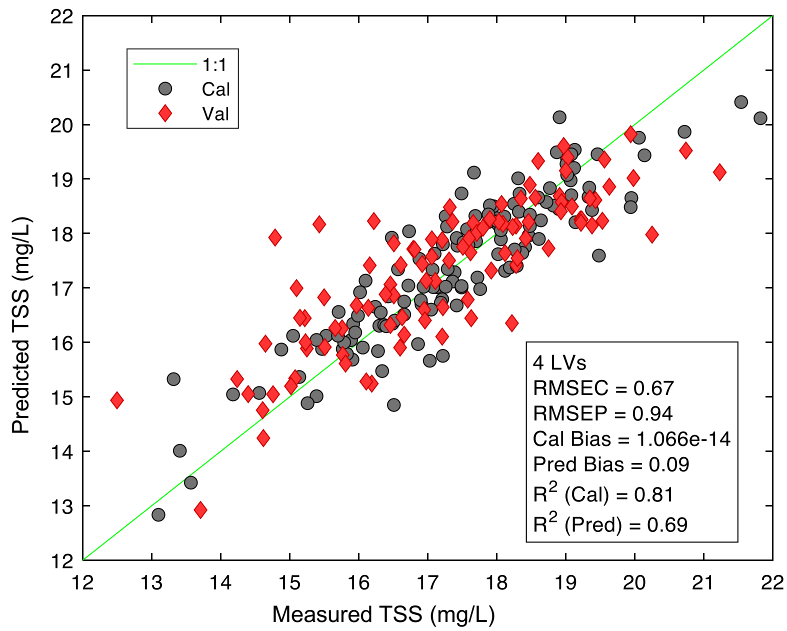

Figure 6. Predictive model for TSS prediction during second year of experimentation.

For the predictive models developed during the second year of experimentation, the same chemometric techniques were employed. However, in alignment with the winery's specific needs, four parameters considered to be the most relevant and representative were selected. Figure 6 presents the PLS model for the prediction of Total Soluble Solids (expressed in degrees Babo instead of degrees Brix), which showed a fairly good error and R^2 in calibration (0.67 and 0.81, respectively), although the predictive performance was not as strong. Further experimentation, including validation of these models, will certainly be crucial to refining the models for the grape variety analyzed.

Regarding the prediction of phenolic parameters, Figure 7 presents the models for predicting anthocyanins at pH 1 (Figure 7a) and total anthocyanins (Figure 7b). Although the results for these parameters were fairly satisfactory, further improvement and validation with additional samples are required.

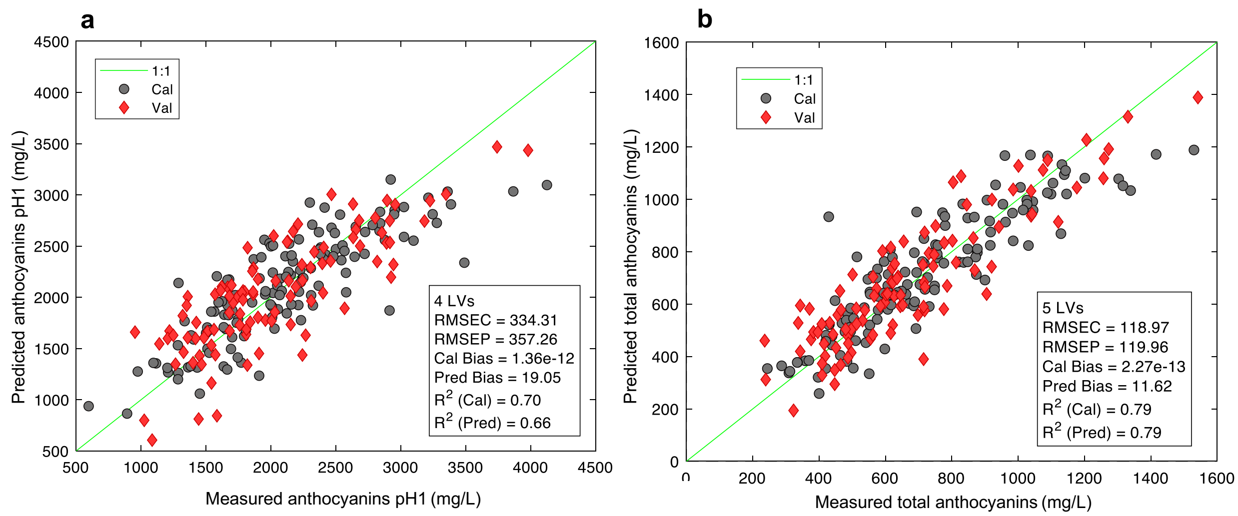

Figure 7. Predictive model for anthocyanins prediction during second year of experimentation.

On the other hand, the PLS model (showed in Figure 8) for the prediction of total polyphenols did not perform well. The model exhibited a relatively high error (266.33 mg/L in calibration and 287.64 mg/L in prediction) and an R² of 0.55 in calibration and 0.45 in prediction.

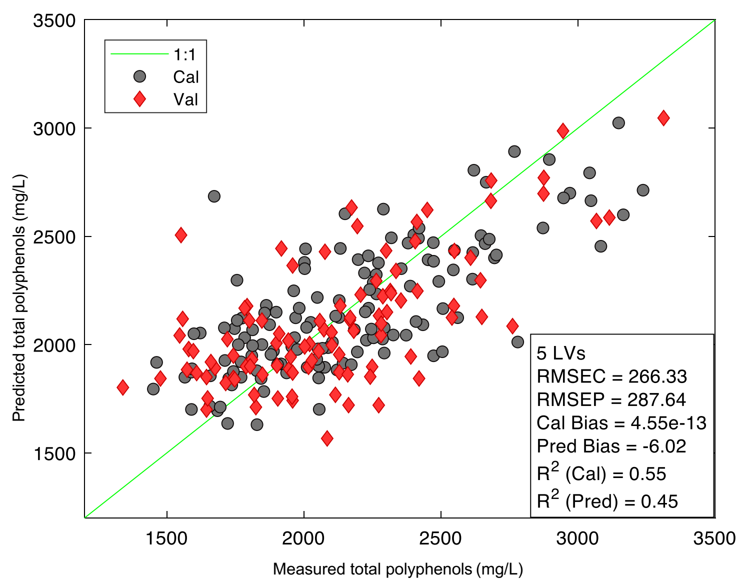

Figure 8. Predictive model for total polyphenols prediction during second year of experimentation.

Conclusions and future perspectives

Once again, it has been demonstrated how vis/NIR spectroscopy can be highly useful for rapidly predicting grape quality parameters without destroying the samples. The use of such instruments has also proven effective on real samples of mechanically harvested grapes, a harvesting technique that is becoming increasingly widespread. The instrument employed is suitable for process applications and, if implemented in the winery, for instance, mounted on a moving mechanical arm, it could be used to take measurements directly from carts containing samples upon consignment. An instrument like this would offer numerous advantages to wineries where various producers deliver their grapes, as it would allow for all quality controls to be conducted at the time of delivery, thereby enabling better management of the grape selection and characterization process upon entry to the winery. This technology could reach its full potential if proximal sensors were used to monitor the quality of the matrix throughout the entire ripening period, not only after the harvest. By enabling continuous monitoring in the field, it would allow for complete control of the raw material, ultimately leading to a final product of the highest quality. By applying vis/NIR spectroscopy, the initial stages of winemaking could be optimized using a technique that is increasingly recognized in the viticulture sector as a green method, fostering the adoption of innovative technologies and smart devices in line with the current industry 4.0 approach.

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4.4 AUTOMATED SYSTEM TO MANAGE FERMENTATION IN AN OENOLOGY 4.0 PERSPECTIVE

The experimentation, similar to that illustrated in the previous chapter, is situated within the wine sector, though it does not involve optical sensors. In this sector, automation is becoming critical due to the need for competitive advantages such as improved quality, increased productivity, and higher profits. By automating certain phases of the winemaking process, resources could be optimized, waste reduced, and real-time production monitoring achieved.

The study presented in this work aimed to analyze a system for the automated management of yeast nutrition during alcoholic fermentation, comparing it with traditional fermentation management methods. The evaluation focused on several factors, and the LCA methodology and cost analysis were applied to assess the performance of both traditional and automated systems. In the context of winemaking, various automated methods have been proposed for online monitoring of the fermentation process. However, the complexity of implementing these strategies on a large scale, due to the need for advanced computer technologies and sensors, has limited their widespread adoption. For this reason, this experimentation laid the groundwork for the development of another low-cost prototype dedicated to liquid matrices (illustrated in chapter 4.5). By successfully integrating optical sensors into automated fermentation management systems like this one, wineries could achieve more precise control over the fermentation process, reducing the risk of quality defects and enhancing the overall consistency and quality of the wine. The ability to monitor fermentation in real-time aligns perfectly with the principles of Industry 4.0, enabling more efficient and sustainable production practices in the wine industry.

PAPER 5: Technological innovation in the winery addressing oenology 4.0: testing of an automated system for the alcoholic fermentation management

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Abstract

In recent years, the use of automated machine tools in wine industry is increasingly gained to simplify and optimize winemaking complying with Industry 4.0 requirements. The aim of this work was to analyze a system for the automatic management of yeast nutrition in alcoholic fermentation, in term of environmental, management and economic performance in comparison with the traditional fermentation management. The automated system is a transportable and easily installable place & start system, equipped with a control unit and rods for the dosage of nutrients and it works with a memory unit in which fermentative kinetics curves are loaded. The curves are predefined or customized according to oenologists needs. Hence, fermentation time, manpower, nutrients, oxygen, water, and energy consumption were evaluated concerning alcoholic fermentation process. The analysis was carried out considering two different Italian wineries with different working capacity. Furthermore, Life Cycle Assessment (LCA) methodology and variable costs analysis was performed.

Overall, the automated system reveals to be a promising investment especially if applied to wineries characterized by high-volume tanks, where scale factor played a crucial role. Nutrients used by the automated system result more expensive but more environmentally sustainable than traditional ones.

Keywords: Industry 4.0, automation, yeast, energy, life cycle assessment, sustainability

Introduction

Automation in viticulture and oenology chains is becoming a crucial key aspect. The necessity of automation in industrial processes is due to the organizations needs to lead towards competitive advantages such as quality improvement, productivity and profits increasing (Caldwell, 2012).

Productivity is directly related to efficient use and management of input resources, which can be improved both in terms of processing rate and waste reduction by automation and optimization. Optimization not only allows the improvement and standardization of the product quality but also its monitoring in a simplified way during the entire process, by sensory applications that work in on-line/real-time mode, to facilitate subsequent correction measures (Caldwell et al., 2009).

Precision viticulture aims to maximize the oenological potential of vineyards and the introduction of new technologies for supporting vineyard management system (Oberti et al., 2013) allows the increase of efficiency and production quality, coupled to a reduction of the environmental impact (Matese and Di Gennaro, 2015). In the oenology process, several automated methods have been proposed for online fermentation monitoring. However, the need of computer technologies and sensors has made these strategies difficult to be implemented in a real scale context. Few industrial fermentations are currently monitored online, but this condition should be overcome soon (Sablayrolles, 2009).

The possibility of a rapid and automated monitoring system of oenological parameters allows winery operators to intervene in a targeted way on the production process, leading effectively to the desired goal. Nevertheless, this is not easy to reach in winemaking, because of the variability of grapes (Alem et al. 2019; Xiao-yu et al. 2007). The implementation of a real-time/online check has allowed the development of automatic systems that can be used remotely for the management of oenological processes (Caldwell et al. 2009).

The increasingly heightened use of both automation and integrated systems is shaping the development of the fourth step of industrialization called Industry 4.0 (Vaidya et al. 2018). This latest industrial revolution is enabled by the development of information and communication technologies (ICT), with its technological basis in intelligent automation (Rojko, 2017). The key to this progress is the combination of plants with great technological potential and the information systems responsible for their operation and control. The terms “connection” and “cyber physical systems” (CPS) mean physical systems (machines and systems) integrated with computers capable to relate to other CPS by identifying the new concept of "smart factory" (Tugnolo et al. 2018). CPSs operate in a self-organized and decentralized way, based on sensor systems applied to obtain data and on actuators to physically influence the process (Stock and Seliger, 2016). Processes implemented with these elements enable even more the profit increase by Organizations, as they adopt strategies such as lean production, just in time production and outsourcing production. In this context, Industry 4.0 succeed in reducing production, logistics and quality management costs from 10 to 30% (Rojko, 2017). This reduction is clear in comparison with Industry 3.0, where the applied technologies can be limited because man is still responsible for actions that not only do not add value but also may be a source of error (Tugnolo et al. 2018). Compared to previous automated productions, systems in compliance with Industry 4.0 are then even more capable of safeguarding and optimizing energy and resources consumption, leading to the development of operating units that are increasingly sustainable from an environmental and an economic point of view (Burritt and Christ, 2016; Stock, Seliger, 2016).

Wine industry is certainly in the process of technological modernization driven by the Industry 4.0 approach (Sá et al. 2020). Fermentation is one of the most critical processes for grape musts. It consists of an exothermic microbial bioconversion reaction, in which the yeast S. cerevisiae from 1 mole of glucose produces 2 moles of ethanol and CO2: $C_6H_{12}O_6$ (180 g) → $2C_2H_4OH$ (92 g) + $2CO_2$ (88 g) +138 kJ (Nardin et al. 2006). The quality of finished product depends on this process.

For a successful winemaking operation, the control of all alcoholic fermentation parameters is essential. The aim is the optimization of the product quality, which is difficult to establish and quantify. Research has shown that the faster the fermentation, the poorer the quality of the wine is, especially for white wines. On the contrary, fermentation which takes too long times increases the risk of off-flavors development and leads to possible damage to aromatic profile of the wine (Sablayrolles, 2009). The fermentation kinetics management is considered a prerequisite to control wine properties (Suzzi and Tofalo, 2018). Despite the technological advances made in fermentation and the energies that oenologists devote to this critical phase to continuously control quality parameters and perform optimization, blocked or poorly conducted processes can still develop with a high economic impact for wineries (Sablayrolles, 2009).

The aim of this work was to analyse a system for the automated management of yeast nutrition in alcoholic fermentation in comparison with the traditional fermentation management approach. The evaluation was carried out considering electricity consumption, use of raw materials, operation time control related to the manpower for the winery workers and related to fermentation cycles. Moreover, the Life Cycle Assessment (LCA) methodology and cost analysis have been applied to evaluate the environmental and economic performance of the traditional and automated systems.

Materials and methods

The automated system

Avaferm® system is a transportable automated, easily installable, and place and start system for automated management of yeast nutrition in oenological alcoholic fermentation. This system is made by HTS Enologia (Trapani, Italy) with patent N° IT201900001239A1 (2019). The system is equipped with a control unit and rods for the nutrients dosage, and it works with a memory unit in which fermentative kinetics curves are loaded. The curves are predefined or customized according to oenologists needs.

The system starts working when fermentation is already activated with must having ethanol values of about 1% vol/vol. At this point, winemaker or winery operators connect the dosing rods to the fermentation tanks. Thanks to the control unit, the starting parameters related to the process are entered. The fermentation is carried out based on desired must (and wine) characteristics to be reached, such as type of must and volume, sugar concentration, readily usable nitrogen, type of yeast, fermentation temperature and oenological objective. Automated system responds by proposing a fermentative kinetic curve, according to a so-called 3Q model (Which, How much, When). The model identifies which organic or inorganic nutrient to add (*Viniliquid, DAP liquid and oxygen*), in which quantity and at what times of the fermentation process.

Experimental plan

For this study, fermentation in real industrial scale winery plants were considered. The unitary operation of alcoholic fermentation was subjected to direct monitoring at the following wineries: Borgo Molino Vigne & Vini Treviso, Italy (BM) with about 10 x 10^6 kg of grapes pressed per year (different varieties for sparkling wine) and Cantina Forlì Predappio Soc. Agricola Coop Forlì Cesena, Italy (FP) with about 60 x 10^6 kg of grapes pressed per year (different varieties, mainly thermovinificated must).

During 2020 grape harvest season, fermentations were set up at the production sites to compare the two methods: traditional fermentation management with manual yeast nutrition and fermentation management using the automated system.

At BM, fermentations of white grape must, Vitis vinifera L. cv. “Glera”, were monitored in cement tanks: one process following the traditional method (tank C18) and two using automated system (tanks C3, C17), with normalized tank volumes at 58 x 10^3 L and filling at 54 x 103 L. The selected fermentations were homogeneous in terms of type of must, type of yeast and duration of fermentation process.

At FP, four fermentations of black grape must, Vitis vinifera L. cv. “Sangiovese”, in stainless steel tanks were analysed in a similar way: two traditional (tank 151 and 158) and two controlled by the automated systems (tank 161a and 161b). In this case, all fermentation tanks had a volume of 153,6 x 10^3 L, containing 135 x 10^3 L of must.

The monitoring operation was carried out collecting data of the following parameters: process timing, manpower, nutrients, oxygen, water, and energy consumption. Moreover, during monitoring activities, interviews were conducted with the wineries staff to collect useful additional information for the study.

All evaluations performed were used as inputs for the comparison between an average tank operated by automated system and an average tank managed according to the traditional method. These criteria were also applied to the LCA methodology.

Data processing methods of the evaluated parameters

Benefits (%) deriving from the use of the automated system or the traditional method for each evaluated parameters were calculated according to Equation 1:

$$R\% = \frac{Value\ min}{Value\ max} * 100;\ Benefit\ \% =\ 100 - R\% \qquad (1)$$

where: value min = minimum average value for automated or traditional method; value max = maximum average value for automated or traditional method.

Consumption (or use) was then related to individual days of usage during the fermentation processes, obtaining comparable graphic trends.

The solid fraction of liquid nutrients formulations (Viniliquid e DAP liquid) was considered as the quantity used. This enables the normalization of the used quantities (active ingredient) of nutrients marketed in the fluid state with the quantities of solid ones (to be dissolved in water). DAP liquid by *HTS Enologia* is composed of bibasic phosphate ammonium in 50% aqueous solution, while Viniliquid by HTS Enologia is a partial autolysate yeast at 60% in aqueous solution. The amount of nutrient used is calculated as shown in Equation 2:

$$Q.DAPliquid\ (kg) = Q.DAPliquid\ (\mathrm{L}) * 0.5\ ;\ Q.\ Viniliquid\ (kg) = Q.\ Viniliquid(\mathrm{L}) * 0.6 \quad (2)$$

The liquid part present in the oenological products is part of water consumption assessment, and it is calculated as shown in Equation 3:

$$H_2O\ DAP\mathrm{liquid}(\mathrm{L}) = Q.DAPliquid\ (\mathrm{L}) * 0.5\ ;\ H_20\ Viniliquid\ (\mathrm{L}) = Q.\ Viniliquid(\mathrm{L}) * 0.4 \quad (3)$$

Energy consumption evaluation

The energy consumed by utilities was calculated as shown in Equation 4:

$$E(kWh) = P(kW) * t(h) * U \quad (4)$$

where: E (kWh) = absorbed energy; P (kW) = nominal power load; t (h) = usage time; U = coefficient of utilization (0.9, 1 in case of nominal power load < 2 kW).

Concerning automated system, data on instantaneous current intensity (A), power supply voltage (V) and timing (h) during active dosages and standby machine periods were registered directly by the system. The total energy (E_{tot}) consumed by the machine was calculated according to equations 5-7:

$$E_{tot}(kWh) = E_{dosage}(kWh) + E_{stanby}(kWh) \quad (5)$$

$$E_{dosage}(kWh) = \sum I_{id}(A) * V_d(V) * t_d(h) \quad (6)$$

$$E_{stanby}(kWh) = I_s(A) * V_s(V) * t_{IDLE}(h)\ /\ 6 \quad (7)$$

with: I_{id}= dosage instantaneous current intensity; I_s = stand-by current intensity; V_d = dosing supply voltage; V_s = stand-by power supply voltage; t_d = dosing time; t_{IDLE} = stand-by time.

The energy consumed by the stand-by system was divided by 6. This divisor is equal to the number of Automated system rods that can dose at the same time.

The stand-by time was calculated according to equations 8:

$$t_{IDLE}(h) = t_{tot}(h) - td_{tot}(h) \qquad (8)$$

with: t_{tot} = total processing time; td_{tot} = total dosing time.

The two wineries worked differently to estimate the energy consumption of the refrigeration systems.

At BM, the heat exchange between must (17°C) and the closed outdoor environment (18-20°C) in which the tanks are located was considered negligible. The heat to be removed from the must is assumed exclusively equal to that developed by fermentation process: 1 g sugar = 0.13 kcal (Nardin et al., 2006). Knowing the sugar concentration of the pre-fermentation must (g/L), the equation 9 was applied:

$$Q_e(kcal) = C_s(g/L) * V(L) * E_f(kcal/g) \qquad (9)$$

where: Q_e = heat to be removed from the evaporator (kcal); C_s = concentration in sugar must (g/L); V = must volume (L); E_f = fermentation energy (0.13 kcal/g).

Tanks are cooled by means of 4 radiant steel plates immersed in the must, in which circulates cold water (plate size = 0.6 m wide x 1.5 m high). A refrigerant distribution efficiency (η_d) of 0.85, a refrigerator efficiency coefficient (ε) of 3.5, a compressor electric motor efficiency (η_e) of 0.9, a mechanical compressor efficiency (η_m) of 0.7 and a compressor usage time (t_c) of 12 h were then assumed.

The energy consumption of the compressor was calculated according to equation 10:

$$Q_eC = Q_e/\eta_d\,;\, w = Q_eC/\varepsilon\,;\, P_c = W/(\eta_e * \eta_m)\,;\, E_c = P_c * t_c \qquad (10)$$

where: Q_eC = heat to be removed, considering the losses of the distribution; W = work done by the compressor; P_c = compressor power load; E_c = energy absorbed by the compressor.

For FP, the same assumptions and calculations were made about the heat produced by fermentation of black grape must (17.5°C), considering in this case a non-isolated system, in which stainless steel fermenters are placed outside and exposed to environmental temperatures, obtained by weather forecast.

To estimate heat exchanges between the must and the environment outside the tank, a simplification was carried out. It was assumed a heat exchange by conduction in a flat wall through the two constituting materials: stainless steel (15 W/m °C) and expanded polyurethane (0.022 W/m °C), with estimated thicknesses of respectively 2 mm and 80 mm. A global heat exchange coefficient and the amount of heat exchanged were calculated based on equations 11:

$$K = \frac{1}{\frac{s1}{k1}+\frac{s2}{k2}};\ Q = KA(T_i - T_o) \qquad (11)$$

where: T_i = temperature inside the tank (must + head space); T_o = outdoor temperature; A = heat exchange area of the tank.

As surface available for heat exchange, the side surface and the base area of a cylinder were considered, as the closest geometric figure to the fermentation tanks (equation 12). The base area of the cylinder in contact with the ground has been excluded as a further simplification:

$$A = S_{lateral} + S_{base}; S_{lateral} = 2\pi rh\ ; S_{base} = \pi r^2 \qquad (12)$$

The total heat that the refrigeration system must remove from FP was calculated based on Equation 13:

$$Q_{tot} = Q_e + Q \qquad (13)$$

Subsequently, as with BM, the energy absorbed by the compressor (Ec) was calculated cumulatively during the entire fermentation processes. At FP it is also necessary to consider a cooling from 20°C to 17°C that took place on the must present in the fermentation base on the first day of the process. The heat to be subtracted (Qr) in this case is equal to (equation 14):

$$Q_r = m * C_p * \Delta T \qquad (14)$$

where: $m\ (kg) = V(l) * \rho\ (\frac{kg}{L})$ or mass in process; ρ= must density (1.050 kg/L); C_p = specific heat (0.855 kcal/kg*°C); ΔT= temperature difference.

Cooling energy absorbed by the compressor was calculated also in this case (equation 10).

For both production sites, the total consumption of the two refrigeration plants was added to those of the other electrical utilities (E) supporting alcoholic fermentation.

Life Cycle Assessment (LCA)

To compare the environmental impact of the automatic management of yeast nutrition in alcoholic fermentation with the traditional fermentation management approach, the Life Cycle Assessment (LCA) analysis was performed in accordance with international ISO 14040 and 14044 standards (ISO 14040, 2006; ISO 14044, 2018).

Functional unit

The comparative analysis implies the same functional unit for the two systems analysed. In this study the functional unit was represented by one average process of alcoholic fermentation conducted with traditional method and automated system. Considering the different capacity of the fermentation tanks, at BM, the functional unit refers to an average process of alcoholic fermentation in a 58 x 10^3 L tank, while at FP an average process of alcoholic fermentation in a 153,6 x 10^3 L tank.

System boundaries

A “gate-to-gate” approach was chosen for LCA analysis. The boundaries of the system are related to the production of all the ingredients necessary for the unit operation of alcoholic fermentation and range from the activation of yeast and filling of fermentation tanks to first pouring, racking and cleaning of the equipment. The grape cultivation activities and the transformation into must were considered out of the scope of the study and therefore out of system’s boundaries.

Definition of the product system

The analysed processes were shown in Figures 1 and 2 for BM and FP, respectively. It is important to highlight that at the start of fermentation (about 1 degree of alcohol) the differentiation between traditional and automated management takes place, respectively highlighted in orange and green in the figures.

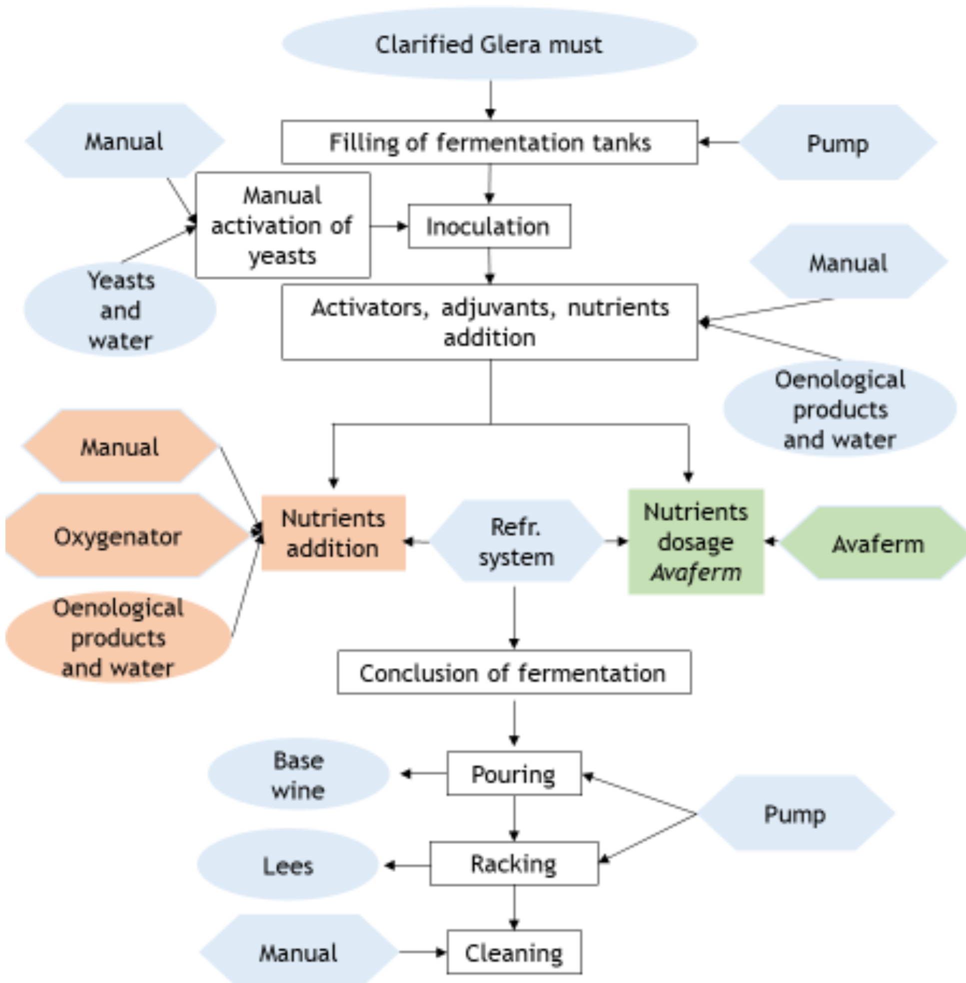

Figure 1. Production process flowsheet at Borgo Molino Vigne & Vini Treviso (BM).

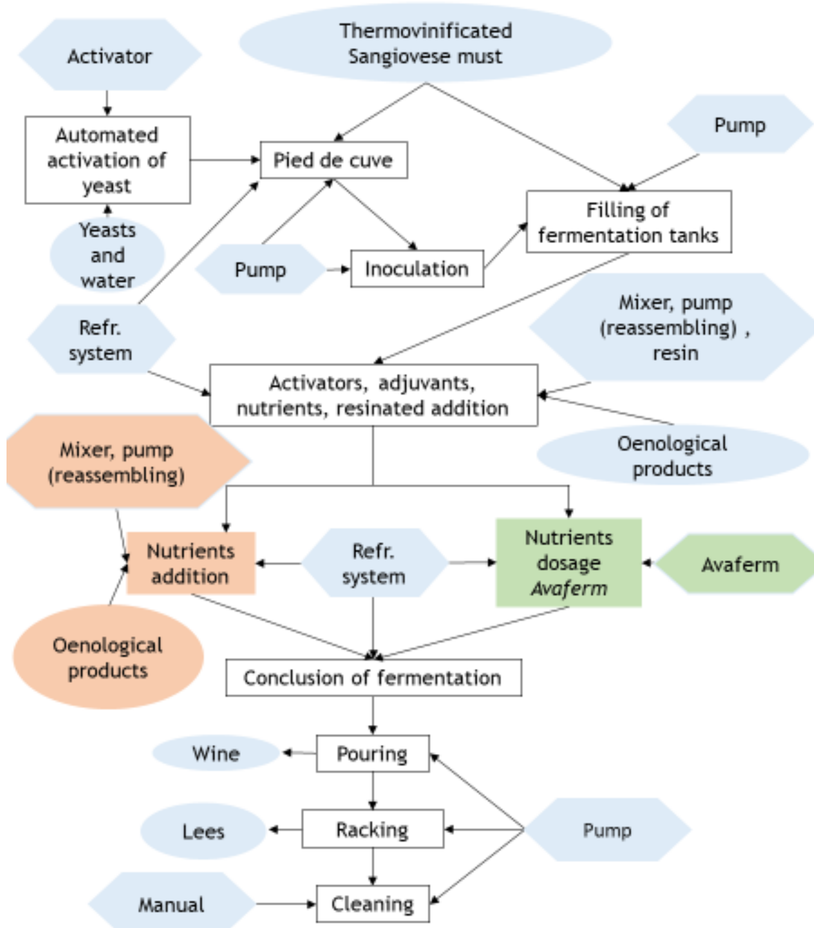

Figure 2. Production process flowsheet at Cantina Forlì Predappio (FP).

Inventory analysis (LCI)

The inventory analysis was carried out with the primary data collected with the monitoring activities and made it possible to identify the resources used at BM and FP for each phase of the production and fermentation processes. In the LCA study, the volumetric dosages of automated system liquid nutrients were multiplied by the values density equal to 1.1 kg/L and 1.3 kg/L, respectively, obtaining the total mass value (kg).

Missing data

The lack of information for some fermentation inputs determined their exclusion from LCA analysis. The constituent materials of the machine instrumentation were excluded from the study due to the lack of detailed information and the possibility of reliable estimates.

In addition, sulphite musts, tannins used at FP (grape seed tannin and tan fermcolor) and stab micro-M (ENARTIS) oenological products were excluded from the study due to the lack of detailed information. Following interviews with FP staff, DESO2, Vitamon CE and Fermaid E oenological products were considered equivalent to Nutristart.

Impact assessment

For the impact assessment, the CML-IA non baseline method (acidification) and CML-IA baseline method (eutrophication, global warming, ozone layer depletion and abiotic depletion, elements, and fossil fuels), water scarcity category based on AWARE method and Photochemical oxidation based on ReCiPe 2008 were used. The eight impact categories are: Acidification (fate not incl.) (kg SO_2eq); Eutrophication (kg PO_4 eq); Global warming (GWP100a) (kg CO2 eq); Photochemical oxidation (kg NMVOC); Abiotic depletion, elements (kg Sb eq); Abiotic depletion, fossil fuels (MJ); Water scarcity (m^3 eq); Ozone layer depletion (ODP) (optional) (kg CFC-11 eq). The LCA was developed using SimaPro software (version 9.1).

Cost analysis

The cost analysis follows the LCA approach, with the same system boundaries, product system and aim of the analysis shifted to the economic field. However, the amortization costs of the plants and the end-of-life costs were excluded, considering only the portion of variable costs weighting on the studied processes. Regarding the inventory phase, all the input materials of the considered system refer to their monetary cost (€). The allocated costs divided among the studied parameters (manpower, oenological products, oxygen, water consumption, energy consumption), which can be considered as variable costs, were summed up for the two different analysed methodologies of fermentation management to obtain a numerical comparison.

Finally, an attempt was made to quantify the advantage given by shorter process times in terms of costs and a payback period for the automated instrumentation. For this purpose, a grape harvest of 60 days, 6 tanks traditionally managed and 6 automated managed were considered, as the automated system has an equal number of rods that can dose at the same time. Furthermore, the extra potential fermentation cycles achievable through automated system were counted as cost savings. The payback period for the two wineries was calculated as shown in Equations 15-18:

$$Cost\ total\ T = Cost\ 6\ tank\ T * Batches\ T\ \left(\frac{tank}{harvest}\right) \tag{15}$$

$$Cost\ total\ A = Cost\ 6\ tank\ A * Batches\ A\ \left(\frac{tank}{harvest}\right) with\ savings \tag{16}$$

$$Savings\ Avaferm = Cost\ total\ T - Cost\ total\ A \tag{17}$$

$$Payback\ (years) = \frac{Cost\ Avaferm\ system\ (€)}{Savings\ Avaferm\ (€)} \tag{18}$$

where the cost of the automated system = 48000€, T = traditional system, A = automated system.

Results and discussion

Fermentation timing

Timelines were drawn to analyse process times for the monitored fermentations (Fig. 3 - 6). Compared to traditional management, the automated system enabled a reduction in fermentation times of 31% at BM and 12.5% at FP, with the possibility of carrying out respectively 1.75 and 0.5 extra fermentation cycles per tank/grape harvest (assumed equal to 60 days). However, this is possible if additional grape material is available and if it is part of the company's marketing choices. Otherwise, the winemaker will have a free tank available to pour batches of wine for the corresponding duration of fermentation, facilitating the winery management. The shorter process timing is due to the ability of the automated system to distribute yeast nutrients in real time when necessary and in the adequate quantities, based on the dosage curves preloaded in the machine and then implemented, keeping the fermentation activity high at the optimum level.

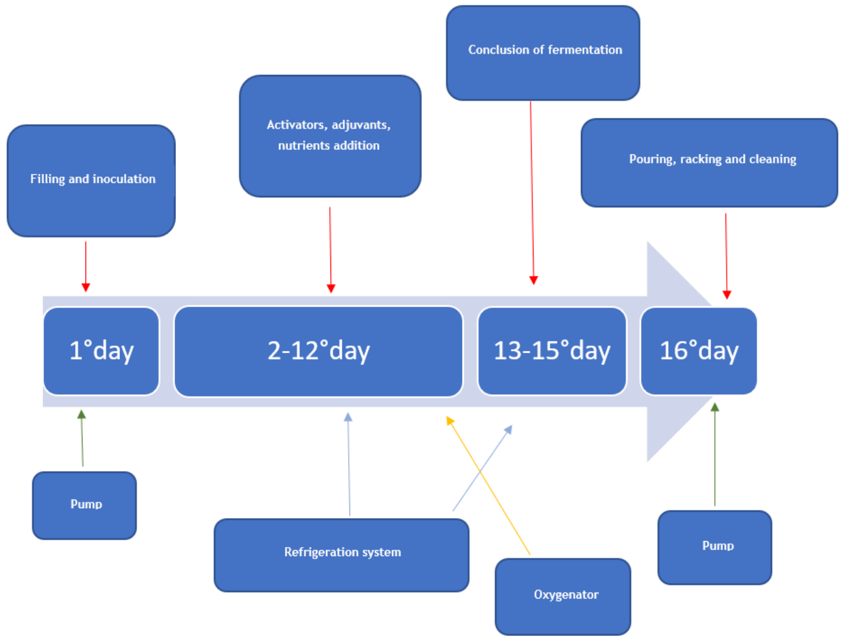

Figure 3. Timeline for traditional system at Borgo Molino Vigne & Vini Treviso (BM).

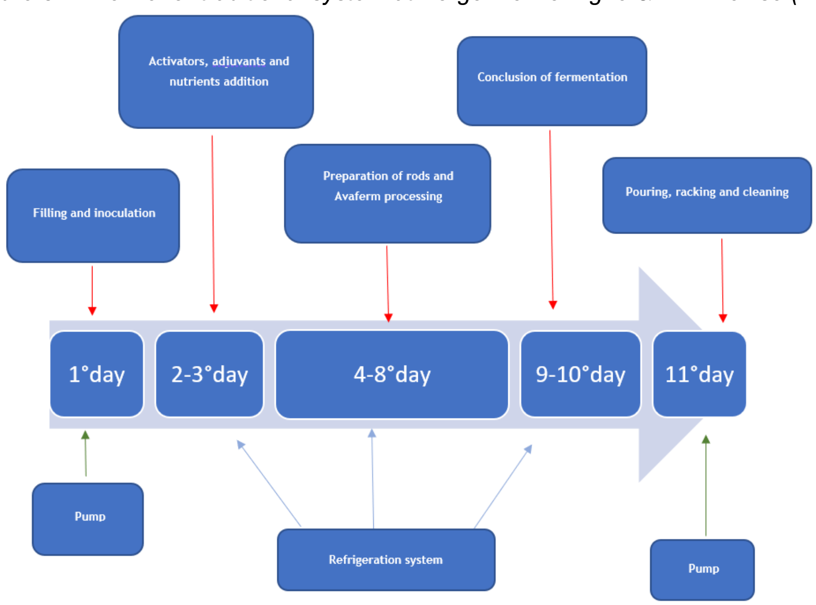

Figure 4. Average timeline for automated system at Borgo Molino Vigne & Vini Treviso (BM).

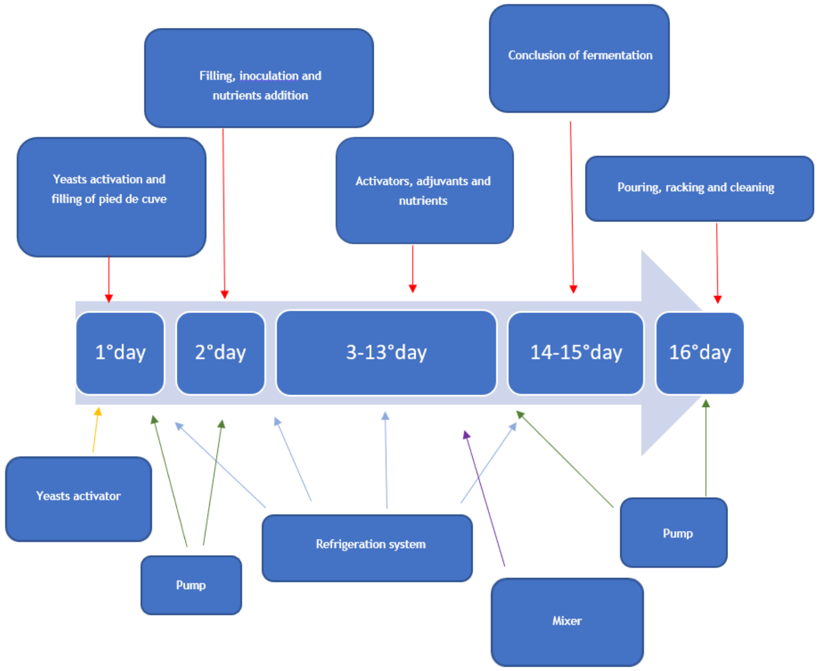

Figure 5. Average timeline for traditional system at Cantina Forlì Predappio (FP).

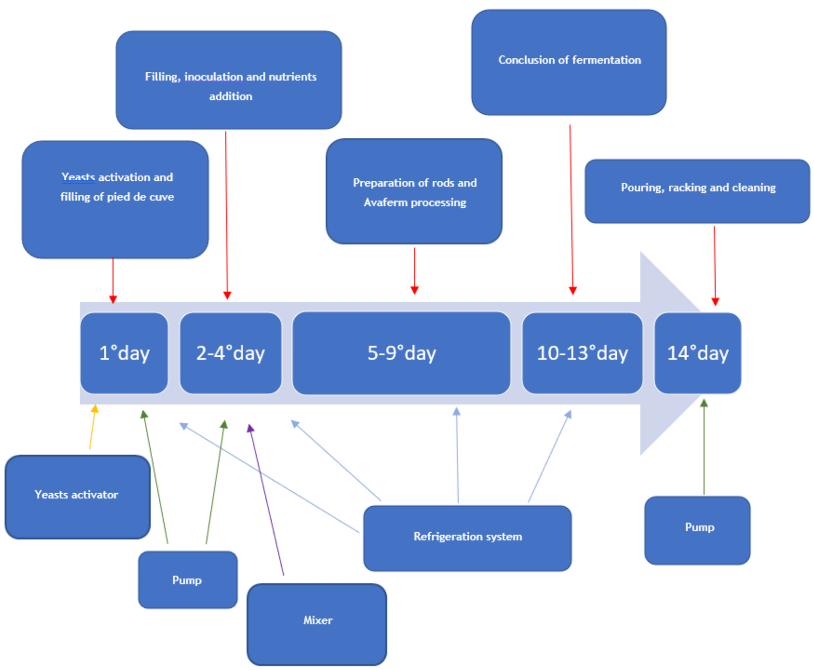

Figure 6. Average timeline for automated system at Cantina Forlì Predappio (FP).

Manpower

Considering the overall fermentation processes it was found that automation allows a reduction in manpower of 18% to BM and 25% to FP. In FP, processes are more complex, and the advantages produced using the automated system tend to be enhanced compared to those obtained for a medium scale winery as BM. Figure 7 shows manpower trends in the two wineries.

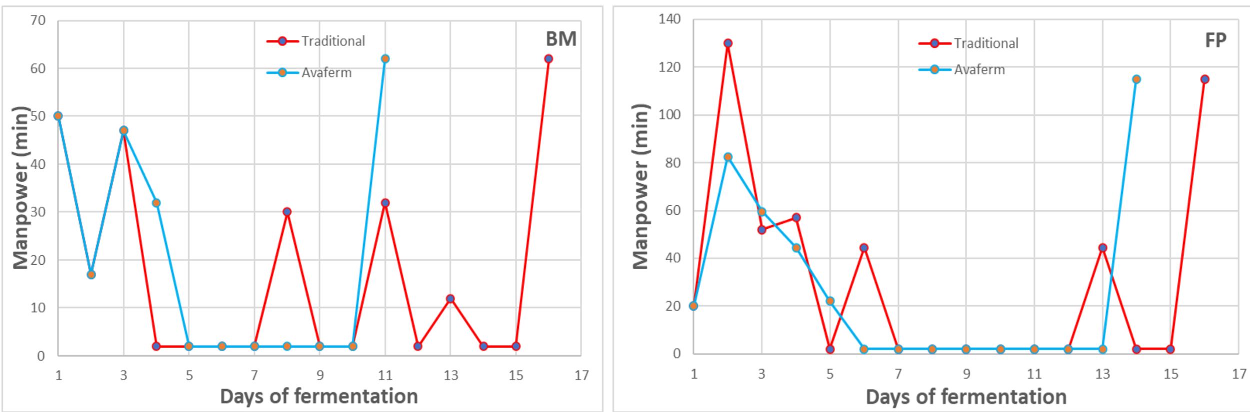

Figure 7. Average trends in manpower: traditional (red) vs automated (blue) systems at Borgo Molino Vigne & Vini Treviso, Italy (BM) (left) and Cantina Forlì Predappio Soc. Agricola Coop Forlì Cesena, Italy (FP) (right).

The lower use of manpower allows to reduce production costs and facilitates winery activities that become less susceptible to human failure, an element of variability that causes a lower capacity of the processes to produce within the specified tolerances. Subjectivity in the operations carried out by the winery operators weighs both on quality of the products and on the profits (Rodríguez-Pérez, 2019).

Nutrients and oxygen

The analysis of nutrients and oxygen use and related trends (Figure 8) during fermentations was crucial because the introduction of nutrients to the must to support and conduct yeast metabolism is the main goal of the automated system. The use of organic and inorganic nutrients was found to be almost similar between traditional management and automated system management at BM. At FP the automation system uses 17.5% more of the considered resource. In the same way, the greater use of resources with automated system management also emerged for oxygen, 32% higher at BM and 53.5% higher at FP. The total amount donated through automated dispensers and reassembling is considered, with reference to a value of 7 mg/L (O_2/remontage), as reported by Ribèreau-Gayon et al 2017.

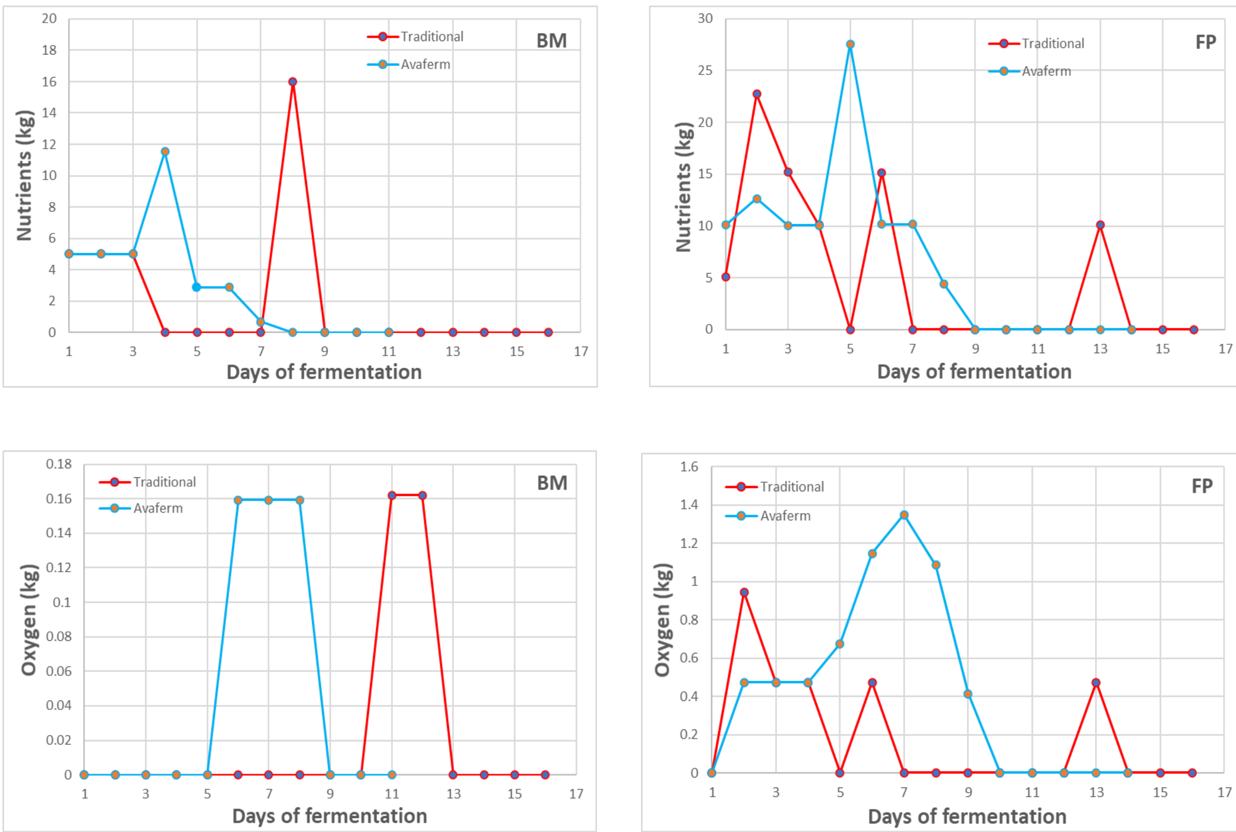

Figure 8. Average trends in nutrients and oxygen: traditional (red) vs automated (blue) systems at Borgo Molino Vigne & Vini Treviso, Italy (BM) (left) and Cantina Forlì Predappio Soc. Agricola Coop Forlì Cesena, Italy (FP).

These values depend by the fact that with the traditional management system it is not always possible to provide the yeast the adequate amount of nutrients: therefore, the microorganism often tends to nutritional deficiency, responsible for any off-flavours and slowed down and sometimes blocked processes (Bell et al 2005; Bely et al 1990; Ribèreau-Gayon et al 2017). These assumptions are supported by shorter process times previously highlighted for the automated system. Finally, nutrient addition carried out with the traditional method is a quite tricky procedure, operators could pay attention to avoid human errors during dosage operation. These difficulties do not occur when automated system is used, guaranteeing to the yeast the essential nutrients for maintaining an optimal metabolic activity, based on the set curves.

Water consumption

Water consumption is an essential factor to be considered in the management of a winery resources: in Europe, wineries use water in average quantities of 5 L for each litre of wine. These amounts are mainly correlated to virtuous behaviour. In general, the use of water resources decreases proportionally with the growth machinery size considered (Lamastra, Trioli, 2014; Trioli et al 2015). Water consumption was lower with the *Automated* system in values of 0.8% and 3.9% at BM and at FP respectively. The savings are due to the unnecessary process of (i) oenological products dissolution before feeding them in the tank (carried out in BM) and (ii) cleaning operation of the fermentation support equipment. These operations are mostly carried out at FP, where the fermentation support utilities are present in greater numbers and where an external mixer is used to dissolve nutrients in a part of must take from fermentation. Nutrients introduced with automated system are already in liquid state and after dosing the feeding rods do not require to be washed.

Energy consumption

Concerning energy consumption, fermentation support utilities (automated system, yeast activator, pumps, and mixers) were first analysed, excluding refrigeration systems which requires specific assessments. Traditional management and the automated one using automated showed the same amounts of energy consumed at BM, while at FP, where the plant is bigger and where greater demand of electrical utilities to support the processes is required, automation implementation saves 4% of energy demand.

In general, the differences between the energy consumption of traditional and automated management depend on the specific organization of winery activities and on the size of the plants. It should also be pointed out that considering only automated consumption in the two different sized wineries, values are always almost the same. As a result, the automated system really suits high volume fermentation tanks. In fact, at BM automated system uses 0.046 Wh/L versus 0.026 Wh/L of FP, with a difference of 28.4% where the volumes involved are higher.

Energy consumption trends of fermentation support utilities are shown in Figure 9. Energy consumption was also assessed including refrigeration systems (total energy). The automated management at BM highlighted a +5% of energy consumed, even if the fermentation cycles were shorter. This positive value is due to a better exponential fermentation activation in the tanks managed by automated system (temperature managed independently from the machine) and to the indoor positioning of the tanks. This allows to neglect the exchange heat between must and outdoor environment and to consider only the contribution given by the fermentation, with the musts all starting from the same sugar concentration. In best conditions, with fermentation started from the same day (equal temperature management), there would have been no differences between the two management methods.

At FP, where tanks are outdoor and exposed to uncontrolled ambient temperatures, the heat removed by the refrigeration system is equal to the heat produced by fermentation and exchanged with the external environment. In this case, the shorter process times enabled the achievement of 9.1% energy savings with the automated system. The refrigeration unit is used for shorter times, depending on the duration of fermentation.

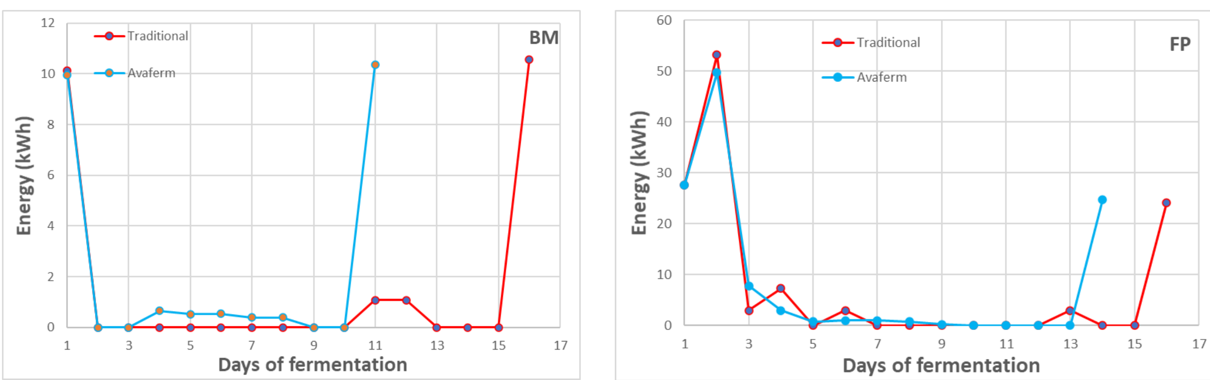

Figure 9. Average trends in energy consumption, excluding refrigeration systems: traditional (red) vs automated (blue) systems at Borgo Molino Vigne & Vini Treviso (BM) (left) and Cantina Forlì Predappio (FP)(right).

Results of life cycle assessment analysis

The comparison between the environmental impact of fermentation carried out with traditional method and with automated system at BM is shown in Figure 10. A better environmental performance for the processes managed by the automation system can be noticed, 1.75% less on average among all impact categories analysed.

The impact categories for which a greater advantage emerged are abiotic depletion fossil fuels (3.29%), ozone depletion (3.14%) and photochemical oxidation (2.05%).

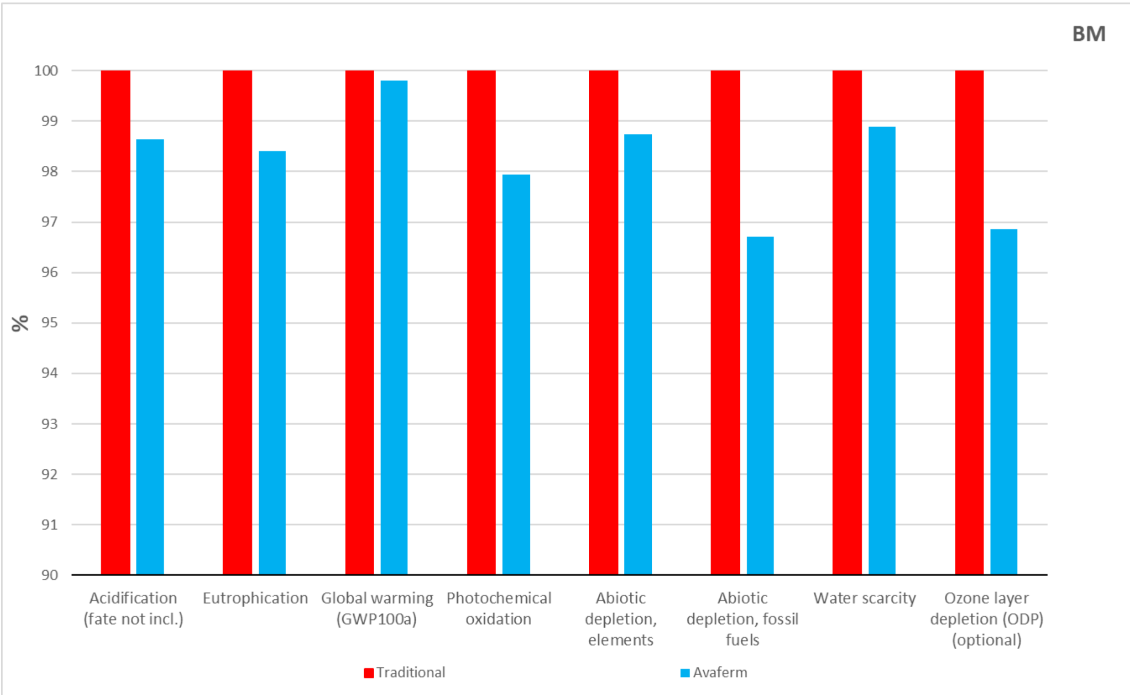

Figure 10. Comparison of the environmental impact: traditional (red) vs automated (blue) systems at Borgo Molino.

Regarding BM, the greater environmental sustainability of automated processes is due to the addition of nutrients which differentiates the two management methods and has a lower environmental impact of 32.7% on average among all impact categories. Furthermore, based on the results presented in “Nutrients and oxygen”, it can be stated that the lower environmental impact is not due to the amount of nutrients but to the use of the right oenological products for yeast nutrition by means of the automated system. Considering the differentiation between the processes, the amounts of nutrients are almost the same between the two management methods, as mentioned above.

Considering only the nutrient addition phase and then the global impact, the recovery of the environmental impact gap between the traditional and automated system is due both to the low weight of the nutrition operation compared to the total impact and to the higher energy consumption of the refrigeration system. However, the latter is independent of the automated system but depend on the different exponential fermentation activation. The environmental impact comparison between traditional management and automated system for FP is shown in Figure 11. Automated management leads to better environmental performance in this winery with a 7.9% less on average among all impact categories analysed.

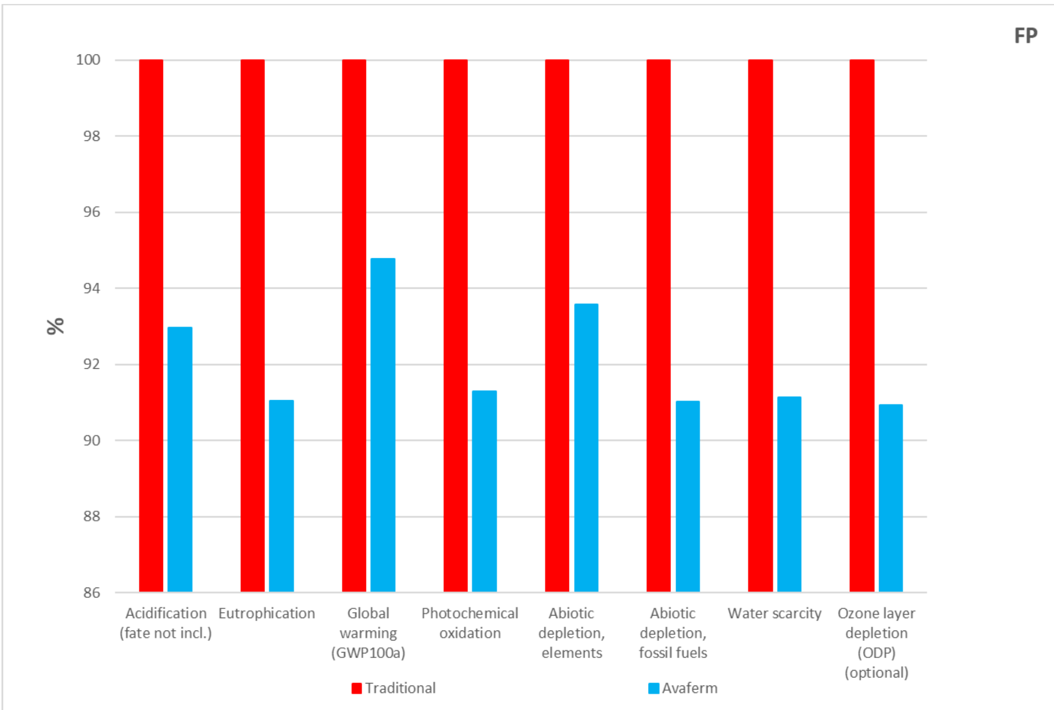

Figure 11. Comparison of the environmental impact: traditional (red) vs automated (blue) systems at Cantina Forlì Predappio.

The impact categories with a greater environmental advantage are ozone depletion (9.07%), abiotic depletion elements (8.97%), eutrophication (8.94%), water scarcity (8.87%) and photochemical oxidation (8.70%). At FP, the lower environmental impact of automated system compared to traditional method is due to the lower energy consumption of the refrigeration system, highlighted by the lower duration of the fermentation process by 12.5%.

Results of cost analysis

Figure 12 shows the results of the variable cost analysis for BM: a +18.8% for automated management compared to the traditional one can be detected.

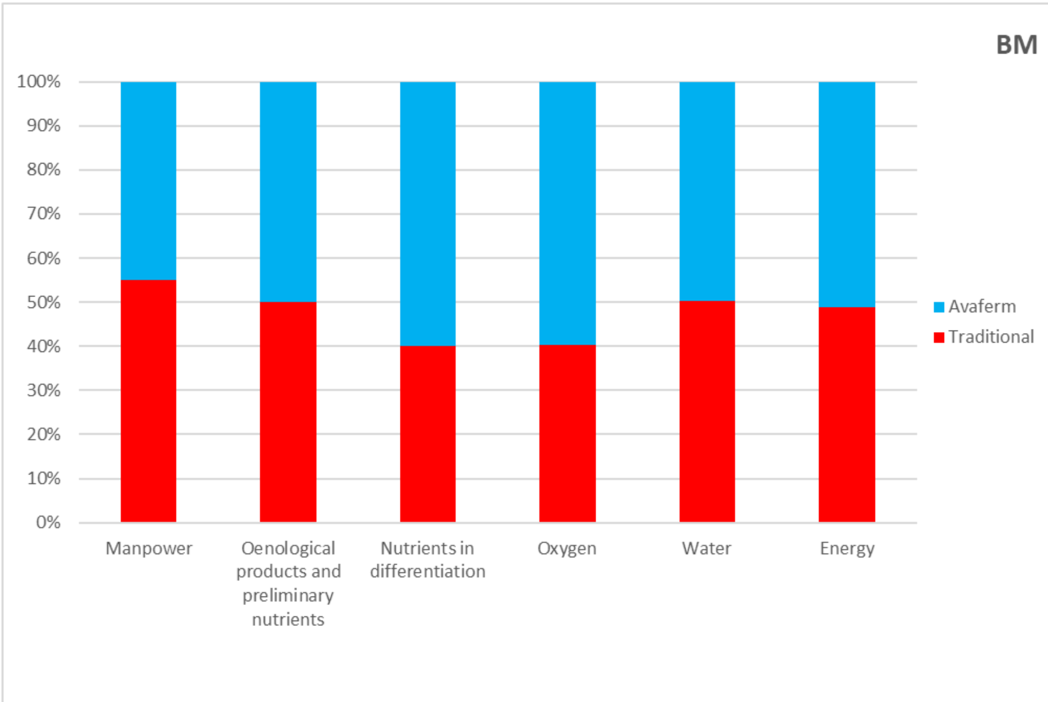

Figure 12. Comparison in percentage for the single variable cost items at Borgo Molino.

The cost analysis considered a comparison based on one fermentation tank and shows that the item with the greatest impact is nutrients in differentiation, with values of 47.69% (351 €) and 58.15% (527.26 €) for the traditional and for automated management respectively. Moreover, oenological products and preliminary nutrients showed also a huge different equal to 36.72% (270.26 €) for the traditional and 29.81% (270.26 €) for automated management. Differentiating nutrients is essential since they refer to the oenological products used during yeast nutrition which enables the distinction between traditional management and the automated system. Cost analysis showed that nutrients used for the automated system are 33.42% more expensive than the ones used in traditional management, having also a greater impact on total variable costs.

The oenological products previously added to the actual nutrition phase are the same and of equal cost and are used in the same amount. In addition, an interesting focus could be done on manpower and energy. Regarding manpower to carry out fermentation process, 6% (44.67 €) and of 4% (36.67 €) could be noticed for the traditional management and the automated one, respectively. Energy consumption accounts for 6.67% (49.08 €) and 5.67% (51.37 €) for traditional management and the automated ones. Manpower cost item expresses the 18% advantage also mentioned in *Evaluation of parameters obtained from monitoring activities*. Electricity costs are mainly guided by the refrigeration system that in BM consumes more with automated system compared to the traditional management.

The FP variable costs analysis results are reported in Figure 13, showing an overall +1.8% for automated management. In this case, the highest cost items are energy, oenological products, preliminary nutrient products, and differentiating nutrients. The highest incidence is due to energy with 66.96% (4006.56 €) and 60.30% (3676.87 €) for the traditional and automated management respectively, derived by the high energy consumption of the refrigeration system and outdoor fermentation tanks. Energy costs are 8.2% lower in the automated system. Oenological and preliminary nutrient products, independent of the management system and related to the characteristics of processed must, showed greater weights in the traditional system (27.07%, 1619.61 €) compared to automated system (13%, 792.98 €), identifying a cost gap equal to +51%.

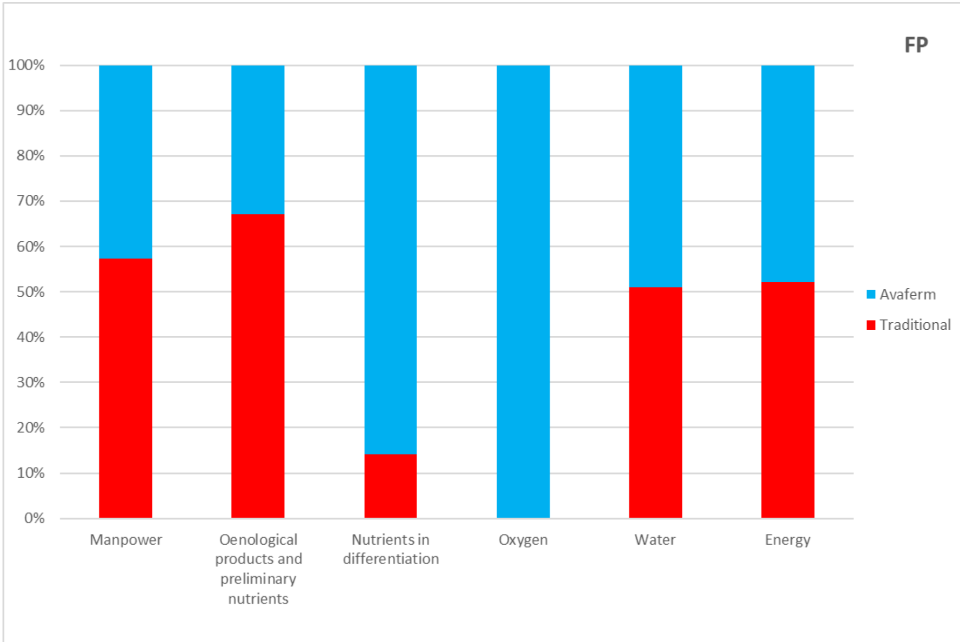

Figure 13. Comparison in percentage for the single variable cost items at Cantina Forlì Predappio.

Opposite results emerged for differentiating nutrients which confirm at FP the BM results, identifying as more expensive the oenological products for automated nutrition. An incidence of 4.22% (252.50 €) with traditional management and 25.19% (1535.88 €) with automated system can be noticed, accounting a plus for this cost item equal to 83.5%. Although its impact on total variable costs is low, it is also worth to highlight a manpower of 1.34% (80.17 €) with the traditional management and 0.98% (59.92 €) with automated system, accounting a difference for manpower costs of 25% to the advantage of the automated system.

Finally, the different process times between traditional and automated management were considered to compare production variable costs in the most realistic way. This led to a payback period value on the costs of the automated system highlighting the investment potential of the analysed instrumentation. The results are proposed in Tables 1 and 2. For BM, a payback period (expressed as savings on production costs) of 8 years could be noticed, the traditional management shows total variable costs of 16561.27 € against 10880.28 € using automated system (i.e. savings equal to 5680.99 €). Regarding FP, a 3-year payback period is calculated, where total variable costs of 134638 € and 118903 € (i.e. savings equal to 15736 €) are shown for traditional and automated management, respectively.

Table 1. Total variable costs: 6 tank traditional vs 6 tank automated system for Borgo Molino Vigne & Vini Treviso, Italy.

Traditional system 6 tank	**Cost (€)**	**Automated system 6 tank**	**Cost (€)**
Manpower	268	Manpower	220
Oenological products and preliminary nutrients	1621.56	Oenological products and preliminary nutrients	1621.56
Nutrients in differentiation	2106	Nutrients in differentiation	3163.58
Oxygen	3.34	Oxygen	4.93
Water	122.97	Water	121.86
Energy	294.46	Energy	308.21
Cost 6 tank	4416.34	Cost 6 tank	5440.14
Cost of unavailability	0	Total cost automated system	10880.28
Total cost traditional system	16561.270		
Batches (tank/harvest) traditional system	N°	Batches (tank/harvest) with savings	N°
	3.75		2.00
Plus batches (tank/harvest) automated vs traditional system			1.75
Savings Automated system (€)	5681		
Automated system cost (€)	48000		
Payback period (years)	8		

Table 2. Total variable costs: 6 tank traditional vs 6 tank automated system for Cantina Forlì Predappio Soc. Agricola Coop Forlì Cesena, Italy.

Traditional system 6 tank	**Cost (€)**	**Automated system 6 tank**	**Cost (€)**
Manpower	481.00	Manpower	359.50
Oenological products and preliminary nutrients	9717.63	Oenological products and preliminary nutrients	4757.88
Nutrients in differentiation	1515.00	Nutrients in differentiation	9215.26

Oxygen	0	Oxygen	48.19
Water	149.60	Water	143.44
Energy	24039.35	Energy	22061.22
Cost 6 tank	35902.58	Cost 6 tank	36585.49
Cost of unavailability	3.74	Total cost Automated system	118902.85
Total cost traditional system	134638.42		
Batches (tank/harvest) traditional system	N°	Batches (tank/harvest) with savings	N°
	3.75		3.25
Plus batches (tank/harvest) automated vs traditional system			0.5
Savings Automated system (€)	15736		
Automated system cost (€)	48000		
Payback period (years)	3		

Conclusions

Conclusions are presented in the form of a SWOT matrix (figure 14) which allows to enhance strengths and weaknesses of the analysed automated system. The differences between the two analysed wineries in terms of size of production sites and number of wine bottles/volumes produced enabled to identify a minimum and a maximum variability of the data within is appropriate to place most of the production companies.

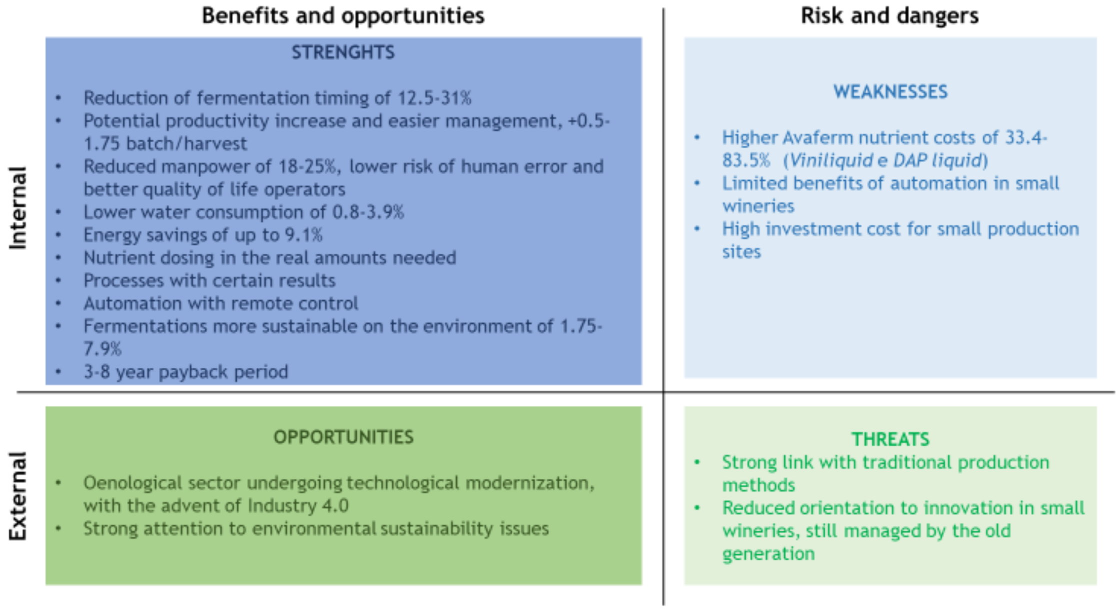

Figure 14. SWOT analysis regarding the adoption of the automated system.

Overall, the automated system reveals to be a promising investment especially if applied to large wineries with high volume tanks, where the advantages given by automation are enhanced. In these production sites, automated system enables the realization of optimized lean productions, through the reduction of all the resources, with exception of nutrients which are more expensive than those traditionally used but more sustainable from an environmental point of view.

Furthermore, where the volumes of fermented must per batch are higher, there is also a lower payback period. From this point of view, smaller production sites may have difficulty in approaching an automation investment and in compliance with Industry 4.0 demands.

The significant reduction in manpower is certainly important, because it leads to minimization of human failure risk, better quality of life of workers, cost savings and better quality of finished product, thanks to the reduction in process variability. This is possible also by means of real-time and remote monitoring of alcoholic fermentation and yeast nutrition, leading to more capable processes than those deriving from traditional management and with clear results. The automated system also allows to obtain more sustainable fermentations from an environmental point of view compared to traditional management.

Finally, automation fits well in a historical oenological context of technological modernization and in a sector increasingly conscious to environmental sustainability issues, although companies (smaller wineries) managed by the old generation are still strongly linked to traditional production methods and not directed at innovation.

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4.5 DEVELOPMENT OF AN OPTICAL PROTOTYPE FOR LIQUID MATRICES

Starting from previous experiments and the feasibility study presented in chapter 4.3, the research group undertook the development of another prototype dedicated to the optical analysis of liquid matrices. The prototype was constructed using a 3D printer and commercial sensors that are available on the market. Several trials were conducted with different liquid matrices, including fruit juices and wine, and various structural modifications were made to the prototype during the process.

As previously mentioned, the prototype is equipped with commercially available components, which include a mini spectrophotometer, a halogen light source, a fan for cooling and maintaining a constant temperature during measurements, and an Arduino electronic board to control the acquisition process. It also requires cuvettes to hold the sample during analysis. The remaining components were produced using additive manufacturing: a main case to contain all the other parts, a holder for the cuvette and optical sensor (Figure 4.5.2), and a cap to cover the cuvette during measurements, minimizing the effects of external light.

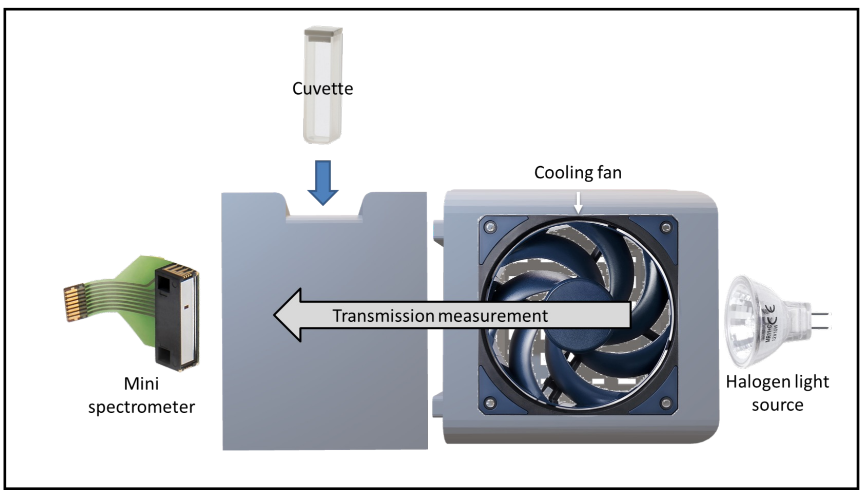


Figure 4.5.1 Principal components of the prototype.

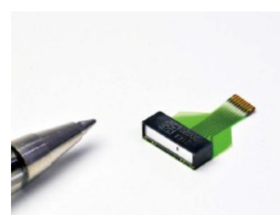
Figure 4.5.2 Main case and cuvette holder of the prototype.

The halogen lamp serves as the light source of the device, passing through the sample contained in the cuvette and reaching the sensor, where the optical signal is recorded. The two sensors cover different spectral ranges and, for simplicity, have been identified as Sensor 1, which operates in the visible range from 340 to 850 nanometers, and Sensor 2, which operates in the Near Infrared (NIR) range from 640 to 1050 nanometers (Figure 4.5.3).

Sensor 1

Spectral range	340-850 nm
size	20.1*12.5*10.1mm

Sensor 2



Spectral range	640-1050 nm
size	11.5*4*3.1mm

Figure 4.5.3 Spectral range and dimensions of the two sensors.

Subsequently, the components of the prototype were housed in an electrician's box to create the final version of the prototype (as shown in Figure 4.5.3). At present, the prototype still needs to be connected to a computer to operate, but one of the next development steps will certainly involve making it autonomous and independent of a computer.

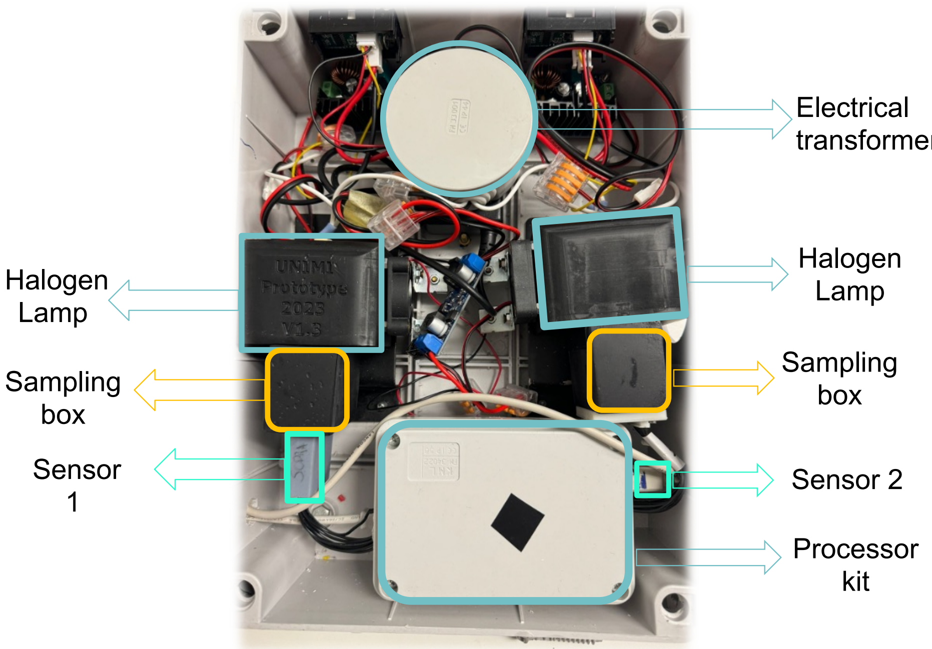


Figure 4.5.4 Final version of the prototype.

This prototype has been employed in two experiments focused on monitoring grape fermentation and has also been used during the research period at Stellenbosch University to monitor the production of craft beer. The device has demonstrated significant potential for monitoring various parameters in different liquid or semi-liquid matrices (ongoing experiments are also investigating olive oil matrices, but the data will not be presented in this work).

In the wine and beer experiments, the data analysis was conducted following the same steps. Data analysis was performed in the Matlab® environment, version 2023b (The MathWorks, Inc., Natick, MA, USA) using the PLS-Toolbox package (Eigenvector Research, Inc., Manson, Washington). The prototype acquires spectra in absorbance, which is measured in arbitrary units that vary for each instrument used. As a first step, the optical data were transformed into percentage transmittance using the white samples (distilled water) acquired as reference. Then, data were analyzed following the various steps characterizing chemometric analysis and its essential function in extracting useful information from spectroscopic optical data. After exploring the raw spectra and the transmittance spectra, Principal Component Analysis (PCA) was performed. This unsupervised technique was applied to identify the principal components or the linear combinations of the original values in exam that at the same time could represent the maximum variance of the samples. Then, in the case of the grape must experiment, predictive models were also developed using the Partial Least Square (PLS) regression technique.

EXPERIMENTATION ON MUST FERMENTATION:

Abstract:
The research aimed to test an optical analyzer prototype for monitoring must fermentation. The compact, low-cost device incorporates a halogen lamp and two commercially available sensors, with custom parts 3D printed at the department. Spectral data acquisition alone is insufficient to measure sugar content. Chemometrics and multivariate statistical analysis correlated optical data with laboratory sugar content measurements to create predictive models. The first experimental campaign calibrated the model using diverse samples, while the second year of experimentation validated it with samples sourced from a wine-producing establishment.
Performance evaluation considers the coefficient of determination (R^2), which should approach 1, and the standard error of estimation (RMSE), which should be minimized. Initial laboratory-scale results were promising, with R^2 of 0.98 and RMSE of 8 g/L for sugar content prediction.
Future work aims to integrate the optical prototype with HTS's Avaferm® system for automated yeast nutrition management during fermentation. This combination could provide an advanced solution for simplifying and optimizing winery operations.

Materials and methods:
During the first year of experimentation, it was decided to test the performance of the prototype for monitoring the fermentation of Ancellotta variety grape samples. A flask experiment was set up to simulate the first 9 days of fermentation. Daily sampling was conducted, which included centrifugation of the samples, optical analyses with the prototype to acquire spectra, and enzymatic analysis using an automatic analyzer to quantify glucose and fructose content. By quantifying residual sugars daily, it is possible to monitor the progress of fermentation.

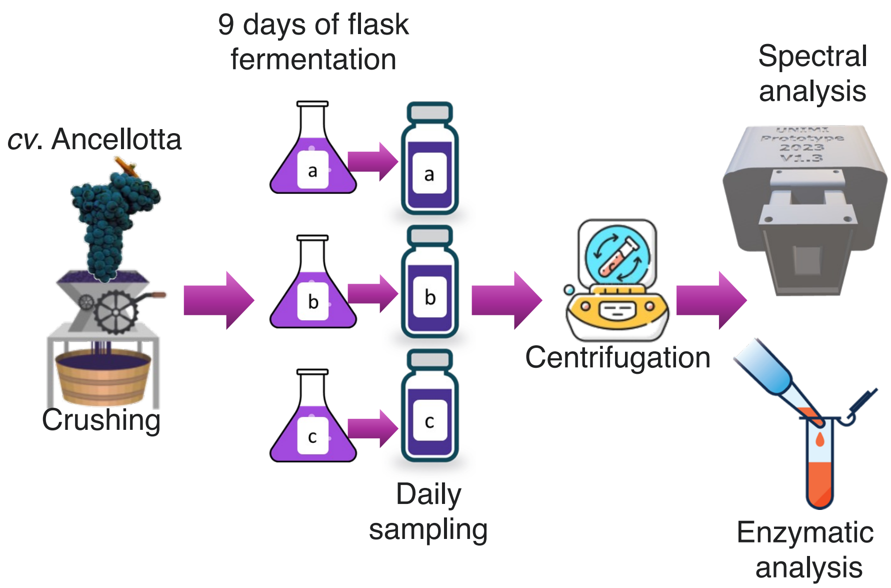


Figure 4.5.5 First year of experimentation plan.

Following the promising results of the first year, a second experiment was conducted using actual samples of red, white, and rosé musts. Two samples were collected in the morning and two in the afternoon, tracking the fermentation of three tanks for each wine type, for a total of 325 samples.

	Red Must			White Must			Rosé Must		
Tank	VR01	VR02	VR03	VB01	VB02	VB03	VX01	VX02	VX03
N° sampling days (fermentation days)	9	7	6	19	12	14	9	9	11
N° samples	**35**	**23**	**18**	65	44	40	26	33	41
tot	**76**			**149**			**100**		

Figure 4.5.6 Samples of the second experimentation.

The samples were frozen and transported to the university laboratories. For analysis, the samples were thawed, centrifuged, and one replicate was analyzed with the automatic analyzer (using the same enzymatic analysis as in the first experiment), while all four replicates were analyzed optically using the prototype.

Results and discussion:

Figure 4.5.7 shows the regression model obtained from the data of the first experiment. The results were very promising, which led to the decision to proceed with the analysis of real samples to validate the analytical method.

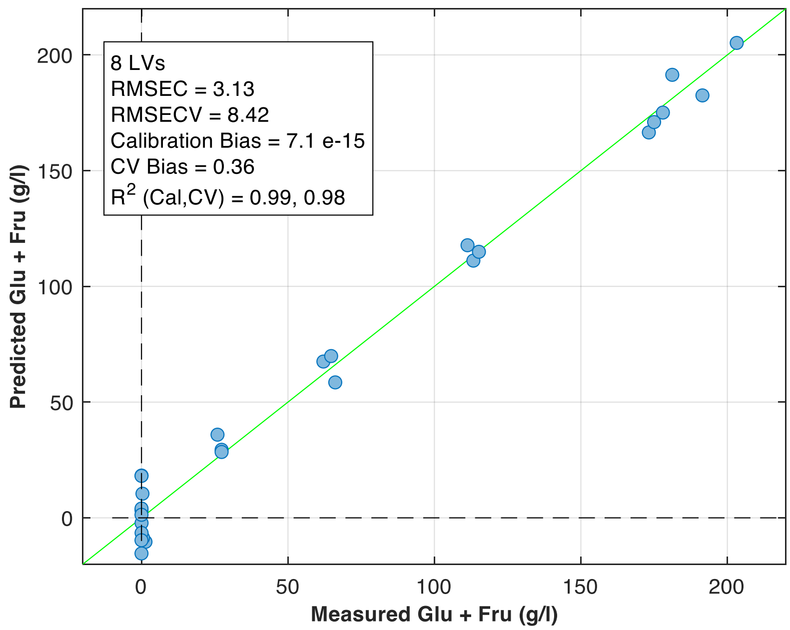


Figure 4.5.7 PLS model for glucose and fructose prediction.

A calibration error of 3.13 g/L and a cross-validation error of 8.42 g/L were obtained, while the R^2 was 0.99 in calibration and 0.98 in cross-validation.

The second experiment involved a larger number of samples, including samples from three different types of wine, to test the performance of the prototype on white and rosé wines as well.

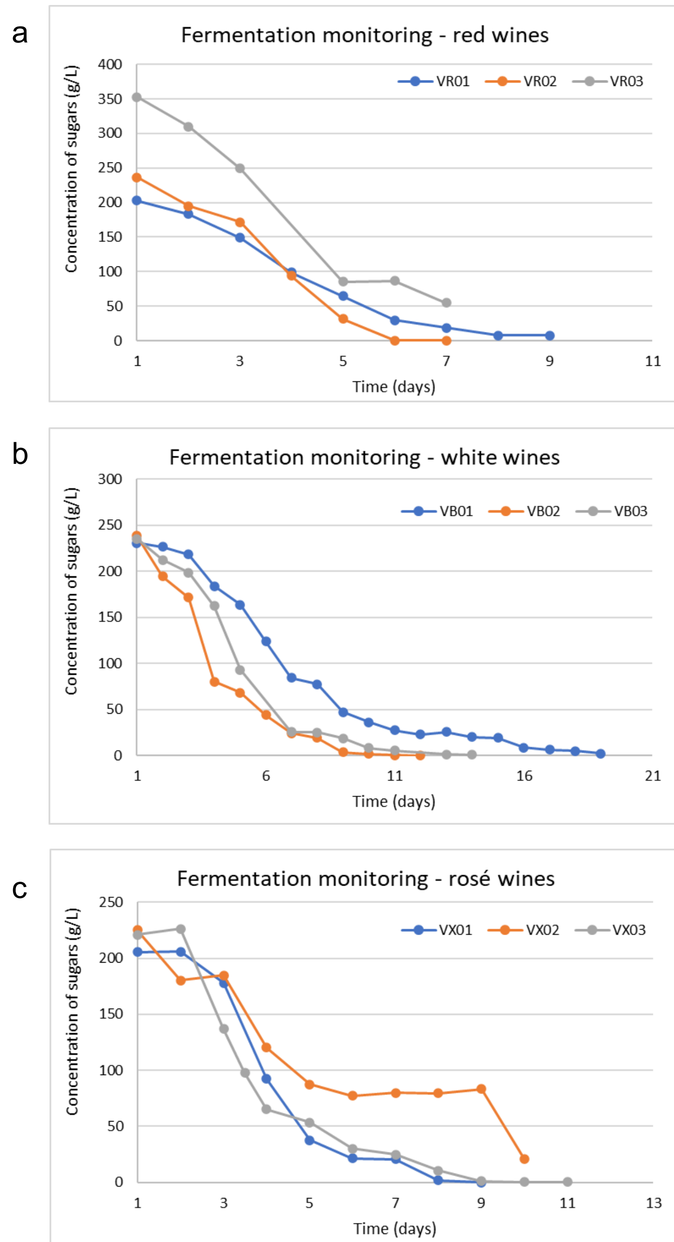


Figure 4.5.8 Fermentation curves of the second experimentation.

Figure 4.5.8 shows the fermentation curves for the different grape varieties and tanks. The figure is useful to represent fermentation progress, identifying variations between tanks, and optimizing the winemaking process to ensure consistent quality and target product profiles.

In this case as well, PLS models were developed by correlating the destructive data obtained from the automatic analyzer with the optical data obtained using the prototype. Table 4.5.1 presents the results of the most effective PLS models for the three grape varieties. Compared to the first experiment conducted under controlled conditions, it is evident that the models built using real samples did not perform as well. The models for rosé wine showed the poorest performance, while the best results were obtained from the models developed from red grape samples.

Table 4.5.1 PLS models performance in sugar prediction.

	Error **RMSEcv (g/L)**	**Fitting** **R^2**
White	20.9	0.94
Red	19.4	0.96
Rosé	34	0.81

Conclusions and future perspectives:

For the prediction of glucose and fructose content, and thus for monitoring grape fermentation, the prototype has proven to be highly promising. It is now well-established that vis/NIR spectroscopy is a reliable technology for the non-destructive determination of many parameters, and in this experiment, it was once again evident how effectively this technology works for sugar determination.

The prototype has been used in a laboratory setting so far, but given the results, one of the next steps will certainly be to simplify it further for direct use in the winery. In the context of Oenology 4.0, implementing an in-line device like this would offer numerous advantages in terms of final product quality, as well as economic savings and reduction of sample waste. Such a low-cost, small-sized prototype, once implemented in-line, could be integrated with other types of sensors and instruments for monitoring wine production (consider the automatic system described in Chapter 4.4).

TESTING OF A LOW-COST OPTICAL PROTOTYPE FOR MONITORING BEER PRODUCTION:

Abstract:

The experimentation was conducted as part of a larger project aimed at developing portable optical prototypes for applications in the agri-food sector, leveraging the proven effectiveness of vis/NIR spectroscopy in this field. The experimentation took place at the pilot plant of the Food Science Department at Stellenbosch University, where three IPA beers were produced using varying recipes, specifically adjusting the amount of hops. Samples were collected at different stages of the production process, centrifuged, and analyzed using a custom-built optical prototype.

The prototype, developed by the Department of Agricultural and Environmental Sciences at the University of Milan (Italy), is equipped with two optical sensors operating in the wavelength range of 340–1050 nm and illuminated by two halogen lamps. The structural components of the prototype were fabricated using additive manufacturing with a 3D printer, allowing for a cost-effective and customizable design.

The results obtained demonstrate the potential of portable Vis/NIR spectroscopy devices for non-destructive and rapid analysis in the brewing industry. By correlating spectral data with chemical and process parameters, this approach can provide valuable insights for monitoring and optimizing production. Furthermore, the portability and adaptability of the prototype suggest promising applications in various agri-food contexts beyond brewing, enabling on-site and real-time quality control. This work highlights the importance of integrating advanced optical technologies into traditional food production systems to enhance efficiency and product quality.

Introduction:

The beer market, being one of the most consumed beverages worldwide, is continuously expanding. As with many products in the food and beverage sector, consumers are increasingly attentive to the quality of beer (de Lima et al., 2023). In 2022, global beer production amounted to about 1.89 billion hectoliters (Statista Beer Production Worldwide) compared to 1.3 billion hectoliters produced in 1998. Nearly half of the beer consumed worldwide (approximately 41% in 2021) is produced by four dominant brewery groups, which occupy the largest share of the entire market (Carvalho et al., 2023).

Beer production is commonly divided into industrial, imported, and microbrewery or craft (Garavaglia and Swinnen, 2018). The latter category is increasingly widespread and is growing to compete in some cases with production by large companies (Nave et al., 2022). The craft beer industry is characterized by small, traditional, and independent production that does not exceed 6 million barrels per year and is not owned 25% or more by a non-craft alcohol industry member, according to the Brewers Association definition (2024). However, the definition of craft production varies considerably depending on the country considered (Cabras et al., 2023).

The brewing industry is an overall mature and competitive sector, renowned for being dynamic and for addressing constantly evolving consumer trends. Furthermore, the industry actively seeks to integrate new and emerging technologies resulting from global research, development, and innovation efforts. Gaining a comprehensive understanding of how to establish efficient and sustainable production processes while maintaining safety standards and premium quality is crucial.

One characteristic that unites craft production is certainly the perception by consumers of drinking more refined beer compared to that produced industrially, a distinctive beer made with innovative yet wholesome ingredients. Beer is a very complex product, composed of numerous aromatic compounds. The raw materials used to produce beer (malt, hops, yeast, and water) and the numerous biochemical reactions occurring during the production process leads to chemical diversity (Schreurs et al., 2024).

Quality control in brewing involves a variety of testing procedures at different stages of production. These tests include monitoring raw materials for contaminants and quality attributes, ensuring proper fermentation conditions and evaluating the final product for flavor, aroma, and stability. Several sensory properties are essential in evaluating quality of beer, such as appearance, aroma, flavor, as well as physico-chemical properties like color, bitterness, or haze formation (Habschied et al., 2023).

Objective testing methods, such as gas chromatography-mass spectrometry (GC-MS) for analyzing volatile compounds and high-performance liquid chromatography (HPLC) for assessing bitterness and color, play a crucial role in standardizing beer production. These techniques provide precise and reliable data, enabling brewers to maintain consistent quality across batches and adhere to industry standards. In addition to chemical analyses, sensory panels are employed to assess the organoleptic properties of the beer. However, sensory evaluation can be subjective and influenced by individual preferences and perceptions. The research is focusing on techniques that can mimic the performance of human sensory organs or otherwise conduct standardized and unbiased controls (Wang et al., 2024).

In craft beer production, there are various technological and methodological solutions that could help producers to monitor proper production. These include the use of specific software for production management, the installation of sensors to monitor temperature, humidity, and pH, the integration of automation and traceability systems, as well as the use of data analysis systems to evaluate the performance of the production process. The widespread use of portable optical data gathering equipment has promoted the attempt to extract qualitative and quantitative chemical information through color (Fulgêncio et al., 2020).

Beer appearance is also an important part of the sensory experience for consumers. Thus, a method for objectively measuring color could be useful (DeLange, 2008). The typical coloration of beer results from various molecules extracted from malt grains and hops during its production (Stinus et al., 2023). Currently, the EBC (European Brewing Convention) method or the SMR (Standard Reference Method) are used to determine color, both of which involve multiplying a conversion factor by the absorbance measurement at 430 nanometers of the sample under analysis (Shellhammer, 2009). These methods can differentiate beers but there are some beers with the same absorbance at 430 nanometers that are visually different (Koren et al., 2020).

It is in this context that this experimentation fits, aimed at the application and testing of a cost-effective optical prototype. The device is a spectrophotometer and was employed to obtain spectral data and calculate the Tristimulus Values of the samples. This type of measurement allows for the representation of the color of transmitted or reflected light and is based on a mathematical representation of how the human eye perceives color. It is a method that has already been used to determine the color of beer and could prove useful because various parameters can be derived from the Tristimulus Values (Smedley, 1992).

Vis/NIR spectroscopy is already widely used in the food field and in the alcoholic beverage sector (Cozzolino and Roberts, 2017) for non-destructive monitoring of several parameters such as moisture, pH, sugar content, and many others (Roberts et al., 2018). Implementing standardized quality controls using this low-cost technology could help breweries to meet regulatory requirements and consumer expectations without conducting lengthy, destructive, and expensive laboratory analyses.

Materials and methods:

The experimentation was conducted at the pilot plant of the Food Science Department at Stellenbosch University. Three IPA beers were produced by varying the recipe, in particular the amount of hops. Samples were collected during the production process, then centrifuged and analyzed with an optical prototype. The prototype used was developed at the Department of Agricultural and Environmental Sciences at the University of Milan (Milan, Italy). It is equipped with two optical sensors operating in the wavelength range between 340 and 1050 nanometers and two halogen lamps used as light sources. The sensors are commercially available (Hamamatsu

Photonics), while the remaining parts of the prototype were constructed using additive manufacturing with a 3D printer.

Samples production and spectral acquisitions

For the production of the wort, and consequently the samples, the various steps of the brewing process were followed, and several samples were taken at each phase of the process, totaling 17 samples per beer as shown in Figure 4.5.9. The first phase of the process (milling) was the only phase where no samples were taken. After grinding the selected malt and wheat, these were mixed with hot water, initiating the mashing phase. During this phase, which lasted approximately one hour, several samples were collected to verify any spectral differences in the extraction of water-soluble components. Typically, during this phase, only temperature, pH, and Brix degrees are monitored, but the introduction of a sensor capable of monitoring the progress of product hydrolysis could be very beneficial for producers (Fox, 2020). After maintaining the wort at approximately 65°C, the temperature was raised to 76°C to complete the mashing phase and begin the lautering phase. Following the transfer of the filtered wort, first hops were added, and the boiling phase commenced. After approximately 45 minutes of boiling, the second addition of hops was made, the boiling phase was completed, and the final production phases began. The last sample was taken at the end of the whirlpool phase, before the beer was cooled and transferred to the fermenter.

The collected samples were immediately placed on ice and, once the production process was completed, they were centrifuged. A benchtop centrifuge (Beckman TJ25, American Instrument Exchange, Haverhill, MA, USA) was used, setting the Relative Centrifugal Force (RCF) to 3000 for 5 minutes. The samples were then transferred to the cuvettes to be read by the prototype.

For each sample, five replicates were prepared, and each replicate was read first with sensor 1 and then with sensor 2. All data were extracted from the prototype, and then Excel matrices were constructed for their analysis.

Sample:	Description:	Phase:
1	Mash after 10 minutes	Mashing
2	Mash after 20 minutes	Mashing
3	Mash after 30 minutes	Mashing
4	Mash after 40 minutes	Mashing
5	Mash after 50 minutes	Mashing
6	Mash after 60 minutes	Mashing
7	Mash after 70 minutes	Mashing
8	Mash ramp 76°C	Mashing
9	End of mash	Mashing
10	End of transfer	Lautering
11	Start of boil	Boiling
12	Boil after 10 minutes	Boiling
13	Boil after 20 minutes	Boiling
14	Boil after 30 minutes	Boiling
15	Boil after 45 minutes	Boiling
16	End of boil	Boiling
17	End of whirlpool	Whirlpoolling

Figure 4.5.9 Samples taken during production and acquired with the prototype.

Results and discussion:

In Figure 4.5.9 the total spectra acquired with the NIR sensor are reported. Without applying any mathematical pretreatment, an initial visual exploration already shows a separation between the three beer samples.

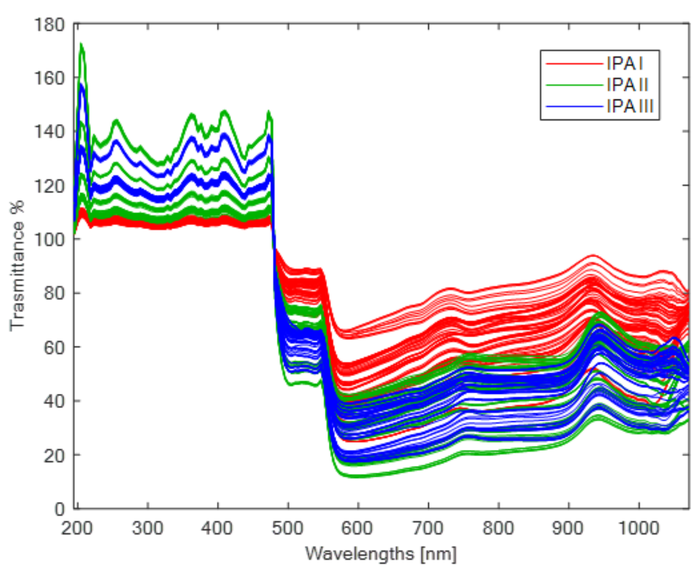


Figure 4.5.9 total spectra of the acquired samples.

The spectra were analyzed using chemometric techniques, and in particular, Principal Component Analysis (PCA) revealed very interesting results. Figure 4.5.9 shows the PCA score plot of the spectra acquired with the NIR sensor. The three IPA beers were produced identically, with only the hop content being varied. The fact that PCA clearly distinguishes between the three different samples highlights the potential of the prototype to differentiate samples that have the same color but different ingredients.

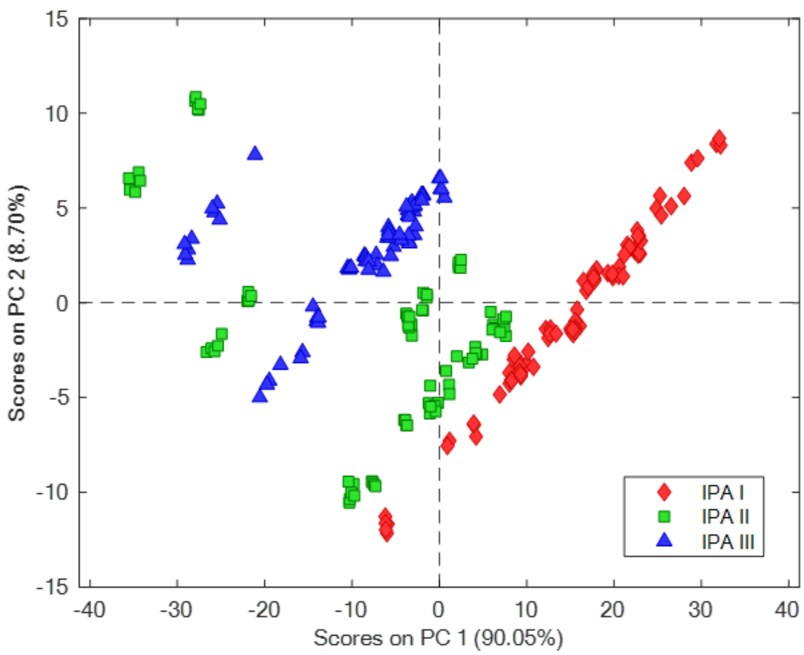


Figure 4.5.10 Score plot of NIR spectra (98.75% of variance explained).

Conclusions and future perspectives:

In craft production or in small to medium-sized enterprises, beer production is often monitored only by color determination and pH measurements. A device like this could prove useful for monitoring various parameters with a single analysis. Further experiments and destructive analyses, correlated with the optical analyses, will be needed to develop predictive models. This initial experiment has shown promise, though the need to centrifuge the samples remains a challenge to be addressed if the optical prototype is to be implemented in-line.

4.6 FEASIBILITY STUDY FOR THE QUANTIFICATION OF ACRYLAMIDE USING VIS/NIR SPECTROSCOPY

The following chapter, which has not yet been published due to confidentiality reasons between the research group and the company B., presents a feasibility study conducted to evaluate the effectiveness of vis/NIR spectroscopy in quantifying the acrylamide content in cocoa biscuit samples. Once again, this technology has proven to be very promising for application at the production line level in industrial food production chains such as the one considered in this experiment. However, further studies and experiments are necessary to validate the predictive models.

PAPER 7: Feasibility study for the quantification of acrylamide in cocoa biscuits using vis/NIR spectroscopy

Alessia Pampuri, Alessio Tugnolo, Valentina Giovenzana, Roberto Beghi and Riccardo Guidetti

Abstract

The cooking process of food products can lead to the formation of process contaminants like acrylamide, a chemical identified by the International Agency for Research on Cancer (IARC) in 1994 as a probable human carcinogen. Extensive research has focused on understanding acrylamide formation, assessing its health risks, and devising strategies to minimize its presence in food. This feasibility study aims to develop predictive models to rapidly estimate acrylamide levels in cocoa biscuits using visible and near-infrared (NIR) spectroscopic techniques. Traditional methods for acrylamide quantification, such as liquid chromatography-mass spectrometry (LC-MS and LC-MS/MS) or gas chromatography-mass spectrometry (GC-MS), are destructive, costly, and time-consuming. In contrast, vis/NIR spectroscopy offers a non-destructive and rapid alternative. The study involved collecting intact and ground cocoa biscuit samples from a manufacturing company, which were then analyzed using vis/NIR instruments: Unity (Hellma®) and Corona® process (Zeiss Group). Chemometric techniques were applied to preprocess the spectra, reducing noise and correcting spectral anomalies. Principal Component Analysis (PCA) was used to explore the data and remove outliers. Partial Least Squares (PLS) regression was then employed to correlate the optical data with acrylamide levels measured by LC-MS/MS. Although the models showed a good linear correlation with the reference method, their accuracy was limited by the small sample size. Future research should expand the sample number and include a wider range of acrylamide levels. Additionally, industrial trials would help evaluate the models' applicability beyond the laboratory. In conclusion, vis/NIR spectroscopy offers a

promising alternative for rapid acrylamide detection in biscuits, potentially aiding future regulatory compliance and enhancing food safety practices.

Introduction

Acrylamide is a small-sized molecule used for various chemical and environmental applications since the last century. It is found in paper, textile, dye, cosmetics, personal care products, and water treatment. This molecule has gained an important place in food science and environmental science due to its carcinogenic effects for humans (Tepe and Çebi, 2019).

Acrylamide is a neo-formed contaminant (NFC) identified in various carbohydrate-rich foods that are prepared through baking, roasting, or frying at temperatures exceeding 100 °C (Lingnert et al., 2002).

The Maillard reaction, which leads to the formation of compounds critical for the physical and sensory properties of food, occurs in products containing carbohydrates and amino acids when subjected to heat treatment. The Maillard reaction is the primary pathway for acrylamide formation in food. It is a complex process involving multiple steps and the formation of various reactive intermediates. Asparagine, an amino acid abundantly found in potatoes and cereals, is the main precursor of acrylamide in food. Acrylamide can form through the reaction between asparagine and the initial products of the Maillard reaction, specifically between a free amino acid and a reducing sugar (Maan et al., 2022).

In 1994, the International Agency for Research on Cancer (IARC) classified this molecule as a Group 2A substance, meaning it is a carcinogen and neurotoxin for humans (Žilić et al., 2020). It is genotoxic and contributes to cancer development by forming strong bonds with DNA (Koszucka et al., 2020).

In 2013, Food Drink Europe released the Acrylamide Toolbox to give manufacturers (including small and medium-sized businesses), national and local authorities brief descriptions of intervention steps that may reduce or prevent the formation of acrylamide in particular processes. The toolbox has been designed to give to small and medium businesses, with limited resources for R&D, individual indications about strategies that could be useful in reducing the generation of acrylamide (Palermo et al., 2016).

European authorities have set benchmark levels for various food categories in Regulation (EU) n. 2017/2158 and among the foods analyzed, the highest contamination levels have been observed in french fries, coffee, and biscuits. For biscuits and waffles, the critical acrylamide limit is 350 μg/kg, with particular emphasis on infant biscuits, where a much lower threshold of 150 μg/kg has been established. Since baby foods can present significantly higher health risks due to infants' lower body weight, manufacturers of baby food must adhere to strict standards and undergo rigorous inspection (Lo Faro et al., 2022).

The application of the guidelines proposed in the Regulation is intended to ensure that acrylamide levels in processed products remain below the reference values. However, the Regulation does not establish legal limits or safety thresholds; it merely suggests reference values with the aim of encouraging all producers to take them into account.

Reducing acrylamide formation during both industrial and domestic food processing is therefore a significant challenge in the food industry. At the same time, in addition to efforts to reduce its formation, it is crucial to measure the acrylamide content in food.

The analysis of acrylamide (AA) in food products is typically conducted using high-performance liquid chromatography (HPLC) or gas chromatography (GC) coupled with mass spectrometry (MS). This detection is often carried out in selected ion monitoring (SIM) mode or through tandem mass spectrometry (MS/MS) in multiple reaction monitoring (MRM) mode, employing isotope-labeled standards (EFSA, 2015).

This project aimed to develop a predictive model for rapidly estimating acrylamide content in cocoa cookies using vis-NIR spectroscopy. This non-destructive and rapid method provides an alternative to traditional laboratory techniques.

This technique is already widely used in the food industry, but this feasibility study was designed to assess the applicability of this method in estimating a contaminant present in very low concentrations in the analyzed foods. The "worst-case scenario" was selected to test the technique's effectiveness because, as mentioned earlier, acrylamide forms as a result of the Maillard reaction. Therefore, a cocoa biscuit (naturally dark in color) was chosen for this reason. Many food companies are moving in this direction, searching for methodologies to rapidly measure acrylamide before actual legal limits are established, rather than just guidelines.

Materials and methods

Sampling

To develop the sampling plan, an experimental design (DOE) was established, taking into account seven factors. During sampling, these seven factors were varied to determine their potential statistically significant influence on the formation of acrylamide in the biscuit samples. The experimental design was implemented at Company B., resulting in a total of 16 different trials. In the experiment, we focused on acrylamide data, disregarding the individual factors.

Nevertheless, the establishment of an experimental design allowed for the collection of representative samples from the production line. Due to confidentiality, the seven varied factors will not be reported. Sampling was conducted over three separate days. The samples were collected at a Company B. plant, where the biscuits were taken directly from the production line, packaged, and immediately sealed. Three replicates of each sample were collected, yielding a total of 45 samples. A portion of the samples was ground immediately after collection. Reference analyses to determine the actual acrylamide content were carried out by an external laboratory commissioned by Company B., while optical analyses were conducted at the University of Milan. Additionally, burnt biscuit samples were collected to provide a reference with a particularly high level of acrylamide. These samples were specifically produced for the experiment and would not normally be intended for sale, but they played a crucial role in constructing the calibration curve of the models.

Optical analyses and instrumentation

The optical analyses were conducted under controlled conditions at the laboratory of the Department of Agricultural and Environmental Sciences at the University of Milan (DiSAA), utilizing two different spectrophotometers on both intact and ground biscuit samples. The samples were analyzed using a full visible and partial NIR spectrophotometer (Corona Process, Zeiss) and a vis/NIR spectrophotometer (Unity, Hellma).

The Corona Process operates within a spectral range of 380 to 1650 nanometers, is equipped with two halogen lamps, and captures samples from top to bottom. In contrast, Unity operates in the range of 680 to 2600 nanometers and features a rotating plate that allows for the sample to be acquired from bottom to top while rotating, ensuring a more representative acquisition of the entire sample. For both instruments, acquisitions were performed in absorbance mode, and at the end of each acquisition, the spectrum of each replicate was automatically calculated as the average of 50 scans to obtain a more representative spectrum and a higher signal-to-noise ratio. For the ground samples, 5 replicates were performed for each sample, with each replicate corresponding to approximately 20 grams of ground sample. In the case of intact biscuits, each replicate did not involve a fixed number of biscuits but rather the double-layer filling of a container to avoid empty spaces that could skew the acquisitions. Two different plates were used for analyses with Corona Process and Unity, as the latter is designed as a laboratory instrument. Using different containers for the acquisitions performed with Corona Process allowed for a more reliable replication of actual industrial production conditions, the environment for which this type of benchtop spectrophotometer is designed.

Acquisitions on intact biscuits were performed on both the top and bottom sides of the samples, as the top surface featured sugar glaze stars. The replicates were conducted as follows (Table 1):

Table 8. Number of replicates of intact biscuit samples.

Instrument	*Biscuit side*	*Replicates number*
Corona Process	Top	3
Corona Process	Bottom	3
Unity	Top	1
Unity	Bottom	1

Using the Unity instrument, fewer replicates were performed on the intact samples because the rotating plate was deemed to provide a sufficiently representative sample even with a single replicate.

Reference analysis

The reference analyses were conducted by an external laboratory commissioned by Company B., providing data on the acrylamide content, which was measured using liquid chromatography coupled with mass spectrometry.

A key aspect considered in the construction of the DOE and the production of burnt samples was the variability covered by the samples. To develop a high-performance predictive model, it is essential to have reference data that represent as much as possible the full range of the parameter under analysis (in this case, acrylamide content). The boxplot in Figure 1 illustrates the variability of the destructive data, with the highest value, represented by one of the burnt samples, being 476 µg/kg, and the lowest value being 138 µg/kg.

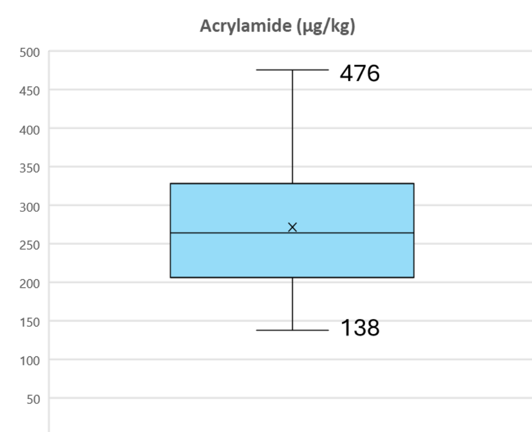


Figure 13. Range of variability in the acrylamide content of the samples.

Data processing

Data processing was conducted following a step-by-step procedure that began with data organization and matrix creation, continued with the visual exploration of spectra, exploratory Principal Component Analysis (PCA), and concluded with the development of predictive models. The data were analyzed in the Matlab® environment, version 2023b (MathWorks, Inc., Natick, MA, USA), using the PLSToolbox package (Eigenvector Research, Inc., Manson, WA, USA).

Spectra were explored through PCA to identify outliers and potential data clusters with similar characteristics. Pretreatment techniques were then applied to the original spectra to reduce noise, enhance resolution, and correct baseline variations. The primary techniques employed included detrending, second derivative transformation, Standard Normal Variate (SNV) normalization, and mean centering.

The spectra and the results of the reference analyses were correlated to create predictive models using the Partial Least Square (PLS) regression technique. After the creation of the models, also known as the calibration phase, a validation phase is necessary to assess their performance. In this case, internal validation, or cross-validation, was employed, primarily due to the limited number of samples available.

To evaluate the performance of the models, several indices were used, including the coefficient of determination (R^2), which indicates the variance explained by the regression model relative to the total variance and should be as high as possible (R^2 values range from 0 to 1). Another commonly used index is the Root Mean Square Error (RMSE), which represents the difference between the observed (or measured) reference values and the values estimated by the model. This measure of error is expressed in the same unit as the parameter being analyzed, in this case, μg/kg. As a measure of error, it should be as low as possible.

Results and discussion

The data were analyzed separately for both whole and ground samples. Through visual exploration of the spectra and Principal Component Analysis (PCA), several outliers were removed, and predictive models were subsequently constructed by correlating the optical data with the destructive measurements of acrylamide. Some illustrative figures are presented, showing the visual exploration of the spectra and the application of preprocessing techniques, highlighting how these methods also visually alter the spectra of the samples.

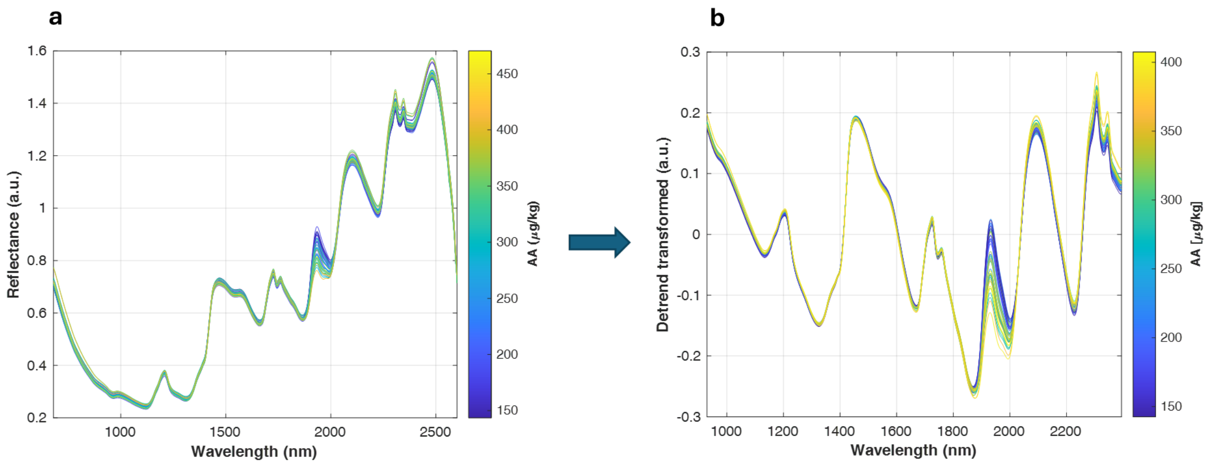


Figure 14. Raw (a) and pretreated spectra (b) of ground samples acquired with Unity.

Figure 2 presents the full spectra of the ground samples analyzed with the Unity instrument. In Figure 2 (a), raw spectra are shown, color-coded according to the acrylamide content, while in Figure 2 (b), the full spectra are displayed after applying the detrending preprocessing, aslso color-coded based on acrylamide content also in this case.

Figure 3 shows the spectra of the ground samples acquired with the Corona Process.

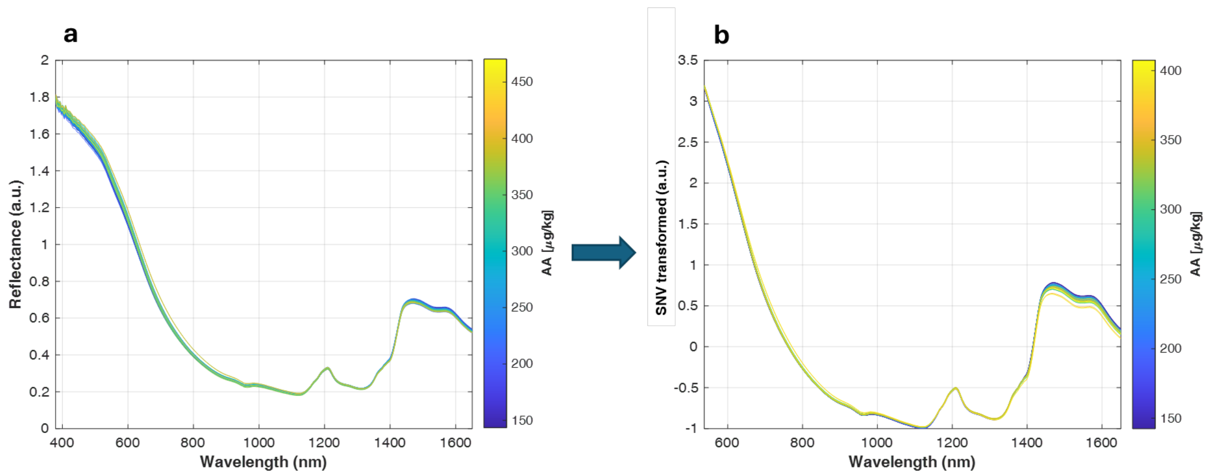


Figure 15. Raw (a) and pretreated spectra (b) of ground samples acquired with Corona Process.

Figure 3 (a) displays the raw spectra acquired using the Corona Process spectrophotometer, while Figure 3 (b) shows the same spectra after applying SNV (Standard Normal Variate) as a preprocessing technique. In both figures, the spectra are colored according to acrylamide content. Figures 4 and 5 present raw spectra acquired from intact biscuit samples, colored based on the acrylamide content of the samples under analysis.

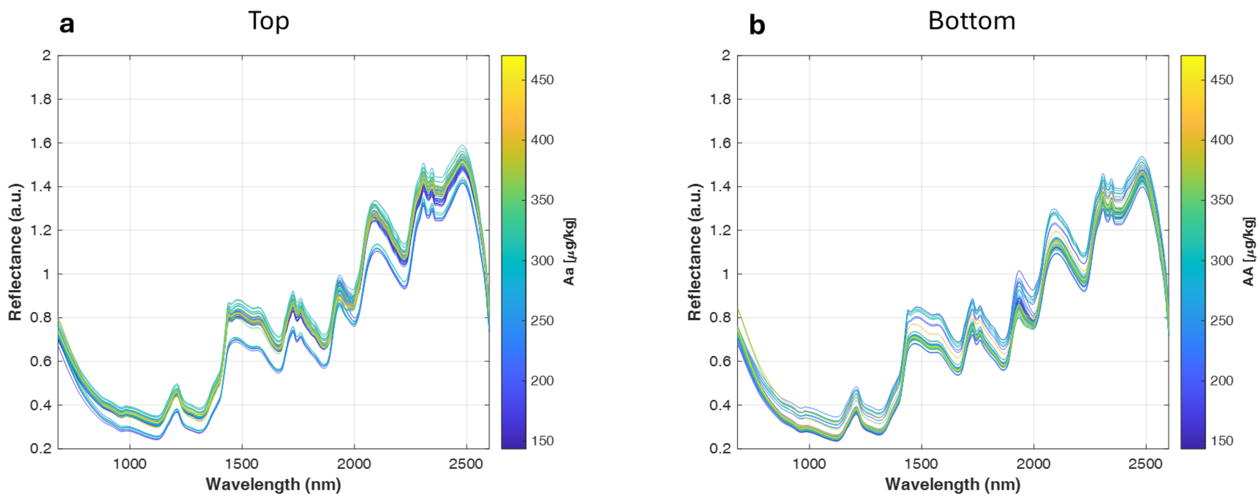


Figure 16. Raw spectra of intact samples acquired with Unity: (a) top side and (b) bottom side.

Figure 4 reports the spectra acquired with the Unity spectrophotometer. Figure 4 (a) shows the spectra from the top side of the samples, i.e., the side with the sugar glaze stars, while Figure 4 (b) presents the spectra from the bottom side of the samples. Similarly, Figure 5 presents the spectra acquired with the Corona Process spectrophotometer.

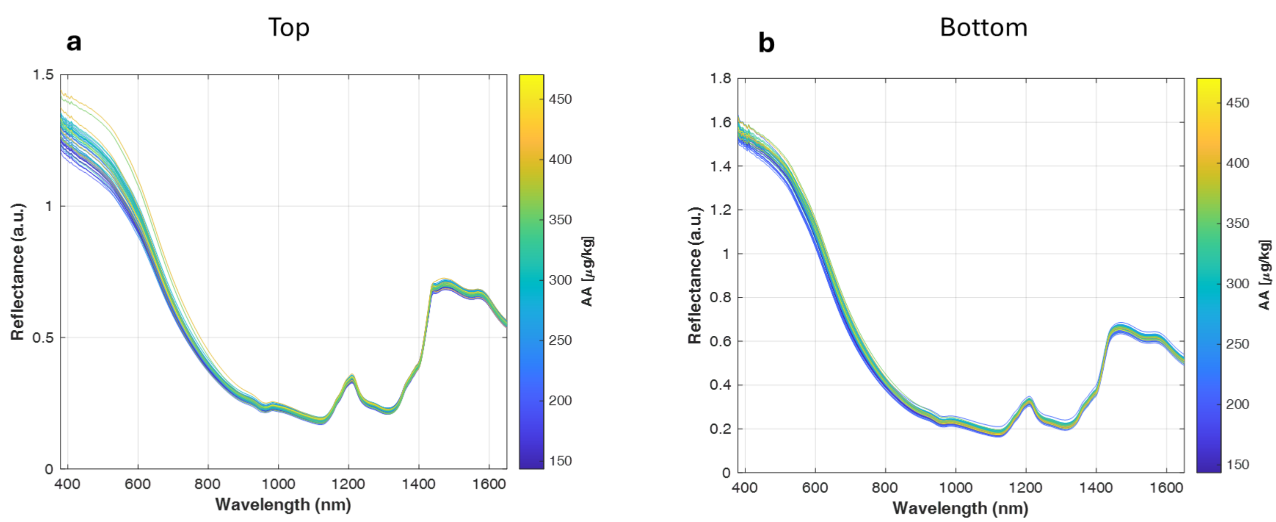


Figure 17. Raw spectra of intact samples acquired with Corona Process: (a) top side and (b) bottom side.

Figure 5 (a) shows the spectra from the top side, and Figure 5 (b) shows the spectra from the bottom side of the biscuits.

<u>Predictive models</u>

Predictive models were developed using the Partial Least Square (PLS) regression technique, applying various preprocessing methods to identify the most effective models.

Figure 6 presents predictive models for the ground samples. The most accurate model was obtained from samples acquired using the Corona Process (Figure 6b), with a coefficient of determination of 0.81 in calibration and 0.70 in cross-validation, and a Root Mean Square Error (RMSE) in calibration and cross-validation of 32.9 µg/kg and 41.76 µg/kg, respectively. The model built with ground samples acquired using Unity instrument also demonstrated good performance, with a slightly lower coefficient of determination in calibration and cross-validation (0.77 and 0.65) and a slightly higher RMSE (36.15 µg/kg and 45.56 µg/kg).

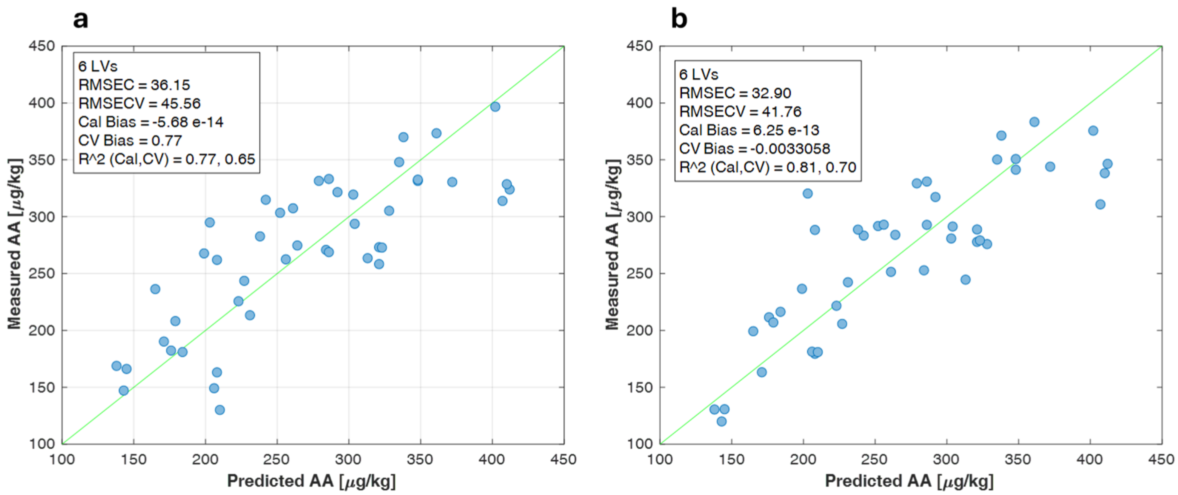


Figure 6. PLS models of ground samples acquired with Unity (a) and with Corona Process (b).

Regarding the PLS models developed on intact biscuit samples, the most effective models, built using data acquired with the Unity instrument (Figure 7) and the Corona Process (Figure 8), are shown.

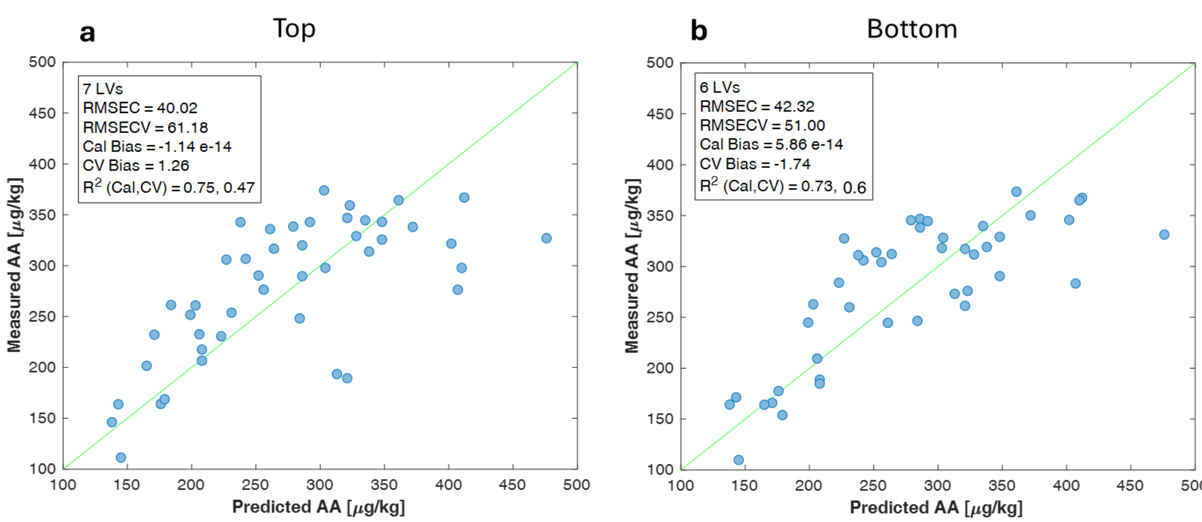


Figure 7. PLS models of intact samples acquired with Unity; top (a) and bottom (b) side of biscuits.

The model that proved to be the most efficient was identified as the one built with data acquired on the top side of the samples (Figure 7a) with a Root Mean Square Error of 40.02 µg/kg and 61.18 µg/kg in calibration and in cross-validation, respectively, and a coefficient of determination in calibration and in cross-validation of 0.75 and 0.47. The model built with spectra acquired on the bottom side of the biscuits also revealed good results with a slightly major error and slightly minor R^2 comparing to the model for the top side.

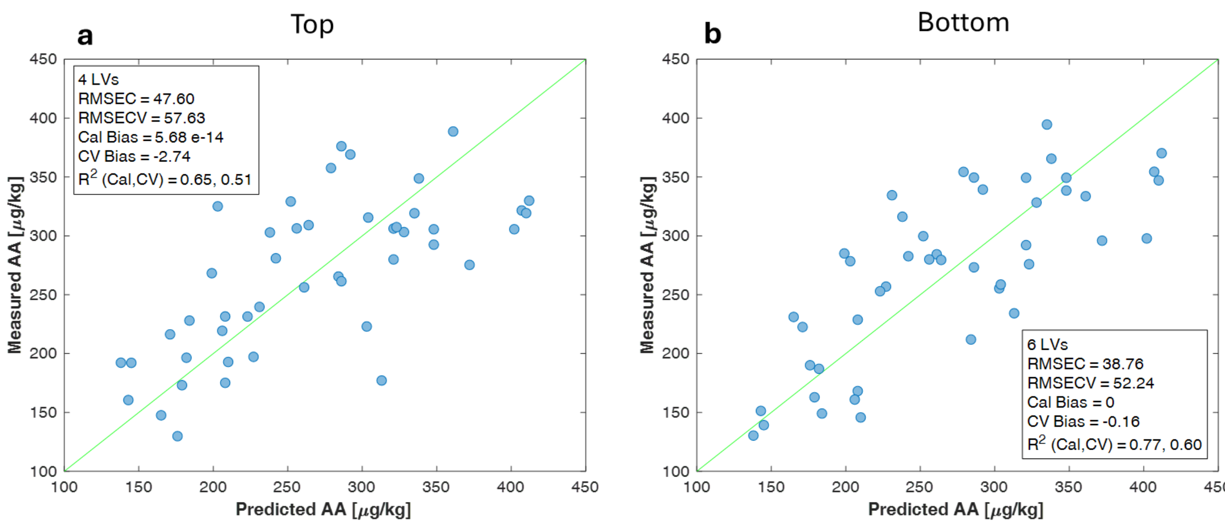


Figure 8. PLS models of intact samples acquired with Corona Process; top (a) and bottom (b) side of biscuits.

The models, still based on the intact samples, built with the data acquired using the Corona Process are shown in Figure 8. In this case, it can be observed that the most efficient model is the one corresponding to the bottom side of the samples (Figure 8b), where the Root Mean Square Error is lower, and the coefficient of determination is higher. This result was expected due to the presence of sugar glaze stars on the top side of the samples. Compared to the Unity instrument, the Corona Process is more suitable for in-line implementation. Using a spectrophotometer like this, mounted, for instance, on a conveyor belt, the samples would only be analyzed from the top side. For this reason, further experimentation is essential to validate these predictive models. The results of the most efficient predictive models are reported in Table 2.

Table 2. Results of the most efficient predictive models and relative pretreatments and number of latent variables.

Sample	*Instrument*	*Pretreatments*	*n° LV*	*R^2 cal*	*R^2 CV*	*RMSE cal*	*RMSE CV*
Ground	Unity	Detrending + MC	6	0.77	0.65	36.15	45.56
Ground	Corona Process	SNV + MC	6	0.81	0.70	32.90	41.76
Intact top	Unity	Detrending + SNV + MC	7	0.75	0.47	40.02	61.18
Intact bottom	Unity	Detrending + MC	6	0.73	0.60	42.32	51.00
Intact top	Corona Process	Derivative 1 + MC	4	0.65	0.51	47.60	57.63
Intact bottom	Corona Process	Derivative 1 + MC	6	0.77	0.60	38.76	52.24

Conclusions and future perspectives

The results obtained suggest that instruments utilizing vis/NIR spectroscopy technology can be employed to estimate acrylamide content in biscuit samples. Several advantages arise from the use of this technology, and the outcomes of the predictive models provide an excellent foundation for further experimentation. First, it will be crucial to validate the models by increasing the number of samples analyzed and including a wider range of acrylamide levels to be able to increase the variability of the models and thus increase their precision. The testing could also be extended to other types of biscuits to assess the performance of these instruments on samples that certainly contain acrylamide but do not exhibit the dark color characteristic of cocoa-based biscuits. The choice of sample was deliberately made to conduct this feasibility study on the "worst-case scenario," i.e., a sample containing acrylamide at parts-per-billion (ppb) levels but also dark in color, which makes estimating this contaminant even more challenging (as previously mentioned, acrylamide is closely associated with the Maillard reaction). Currently, there are no legal limits regulating acrylamide content in food, but such limits may be introduced in the future. Therefore, it is advisable for companies and the broader production sector to move in this direction to identify the best method for ensuring rapid and reliable acrylamide detection, in a short time and without sample destruction. Another potential development could involve the creation of classification models rather than quantification models for acrylamide. This would allow real-time identification of compliant and non-compliant samples, once a threshold recognized as acceptable by the company is established, before it is replaced by legal limits. It is thus essential to implement this type of instrument in-line, where non-destructive optical analysis can monitor various parameters with a single device, creating an advantage in terms of product quality, food safety, and waste reduction.

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4.7 DEVELOPMENT OF A PROTOTYPE FOR MEDIUM-SIZED SOLID MATRICES

Building on the initial trials conducted with the first prototype, as detailed in Chapter 4.1, subsequent structural modifications were introduced to expand the device's applicability to larger agricultural matrices. The initial prototype, primarily designed for small matrices such as grapes, proved effective; however, the need for non-destructive analysis of larger samples, including apples and pears, necessitated further adaptation.

A third prototype was developed, integrating three commercial sensors with 6 wavelengths each (Figure 4.7.1) and utilizing additive manufacturing techniques through 3D printing (Figure 4.7.2).

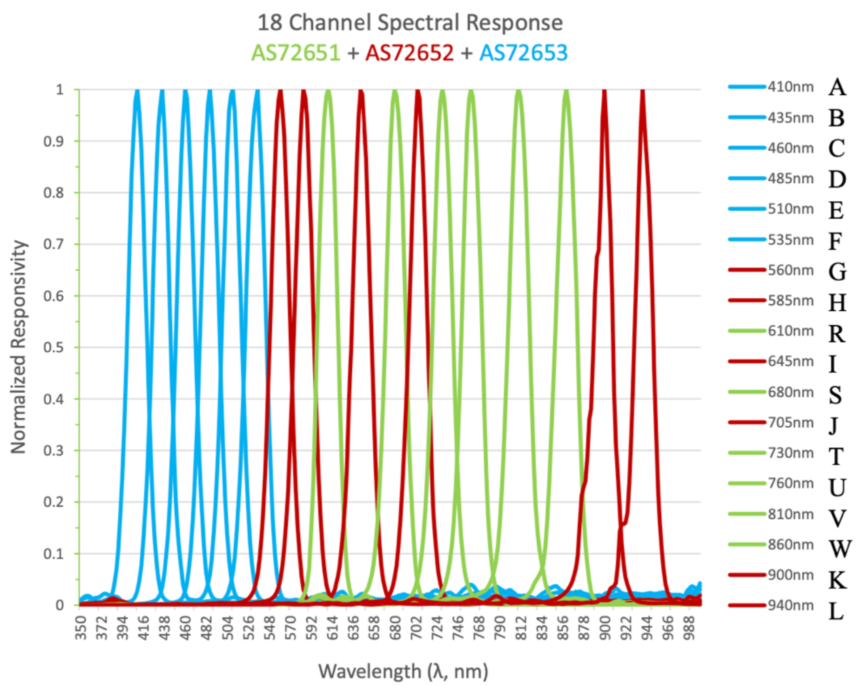

Figure 4.7.1 Spectral Response and wavelengths of the three sensors used.

Figure 4.7.2 3D printed parts of the prototype.

This enhanced version was specifically designed to accommodate the larger size and structural properties of new matrices, while maintaining the precision and non-invasive nature of the analytical process. The promising preliminary results of this research underscore the potential for real-time quality monitoring of larger fruit samples. The true novelty of this project lies in the planned upgrades to the prototype, which aim to empower operators to oversee entire batches, accurately classify products, and provide detailed quality information to consumers. The implementation of such devices holds significant potential to revolutionize the management of harvest and storage processes, thereby optimizing the quality of agricultural products for consumption. The proposed prototype represents a key advancement toward improving operational efficiency in the agricultural sector.

PAPER 7: Application of a simplified optical prototype for maturation and senescence monitoring of medium-sized fruits

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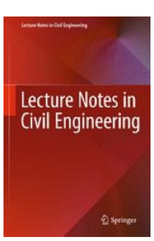
Abstract. The study aimed to test a cost-effective portable optical prototype designed to monitor the ripening and senescence of medium-sized fruits. The device is in a pre-prototype stage and is simplified compared to full-range commercial instruments, operating with 18 wavelengths in the visible-Near-Infrared range (410 nm to 940 nm). The prototype was tested on Fuji apples (Malus domestica 'Fuji'), Passacrassana pears (Pyrus communis 'Passe Crassane'), Dottato figs (Ficus carica L.), and kiwis (Actinidia deliciosa) to analyze their ripening process from early stages to rotting. The challenge was to assess the applicability of the prototype on fruits with different exocarp characteristics such as thickness, color, and texture. A commercial device was used as a reference. Optical data were collected on various sampling days to evaluate changes in the optical features. The ultimate goal will be to correlate optical data with destructive analyses regarding parameters such as Total Soluble Solids (TSS), acidity, and pH to create predictive models capable of predicting these parameters, as well as providing more precise information on the ripening and senescence of these products. The preliminary qualitative results are promising, indicating potential for real-time monitoring of interesting features of the fruits studied. While vis/NIR spectroscopy is already widely applied in these fields due to its numerous advantages, the innovation here lies in advancing the prototype to manage batches, classify products, and potentially educate consumers. Implementing these new devices could improve and simplify the management of harvesting and storage phases, optimizing the quality of agricultural products intended for consumption. The upgrade of this prototype could offer significant benefits for batch management and product classification, enhancing both production efficiency and consumer education.

Keywords: vis/NIR spectroscopy, prototype, 3D printing, predictive models, ripening.



1. Introduction

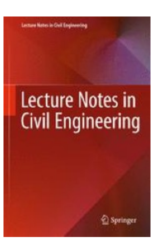
Innovation, efficiency, and sustainability have been propelled forward in the agricultural and food industries in recent years by the incorporation of cutting-edge technologies. Among these novel technologies, visible/NIR spectroscopy has shown itself to be an effective instrument for agricultural product analysis (Zhang et al., 2021).

This technique has the potential to transform how we monitor and manage fruit quality, providing effective solutions to address challenges in the agri-food sector, especially in reducing waste and optimizing resource use. Spectroscopy entails examining the interactions between matter and electromagnetic radiation. Within agriculture, it is a flexible tool for evaluating the physical and chemical characteristics of various crops. While the application of spectroscopic methods in agriculture is not a recent development, technological advancements have significantly enhanced its use, making it an essential tool for precision farming and quality assurance (Cavaco et al., 2022).

Moreover, new miniaturized systems are now available with performance comparable to laboratory-grade optical instrumentation (Pissard et al., 2021). This aspect marks a significant advancement in the field of spectroscopy, particularly due to its simplified design and potential for real-time monitoring, making it especially useful for supply chain operators and potentially for end-users in the future.

One of the strengths of spectroscopy lies in its ability to acquire a wide range of data from the interaction of light with matter without destroying the samples. The features of the device, operating in both the visible and near-infrared regions, allow for comprehensive readouts of the analyzed fruits and related parameters, without only considering the color changes in the peel (Ncama et al., 2018).

The general objective is to achieve a thorough description of the maturation process, from its early stages to its eventual decay, using a relatively simple tool. The application of spectroscopy in the agricultural domain has already gained significant traction due to its numerous advantages. It enables non-destructive testing, meaning that the quality and characteristics of fruits can be assessed without compromising their integrity (Stella et al., 2015).



Authors in 2021 already designed and tested a cost-effective, user-friendly optical device specifically developed for small berries capable of determining the optimal harvest date based on grape ripening. The pre-prototype incorporating sensors for spectral identification in the visible and SW-NIR ranges (450 nm to 860 nm), was tested on Nebbiolo grapes. Optical data were correlated with traditional lab analyses of soluble solids content, titratable acidity, extractability of anthocyanins, and pH (Pampuri et al., 2021).

In this case, the study aimed to test a cost-effective, portable optical proto-type for monitoring the ripening and senescence of medium-sized fruits using 18 wavelengths in the visible/near-infrared range. Tested on several fruit samples, the optical data, coming from the device, were used for a preliminary test of screening capabilities. The prototype could improve batch management, product classification, and consumer education, optimizing agricultural product quality.

This non-invasive approach is critical for preserving the market value of the produce and ensuring that the end consumer receives a product of optimal quality. In addition to the immediate benefits of real-time monitoring, the availability of rapid optical instruments aims to enable operators to manage batches effectively, classify products, and even more a future perspective to educate consumers on the quality of the fruit they purchase. The integration of spectroscopy with food management practices could represent a turning point in efforts to reduce waste (Roy and George, 2020). By providing information on the state of ripeness and quality parameters, this technology could equip stakeholders with the knowledge needed to make informed decisions regarding harvesting, storage, and distribution. This, in turn, could significantly reduce the high levels of waste in the food supply chain.

2. Materials and methods

During the experimentation, a cost-effective portable optical prototype specifically designed for monitoring the ripening and senescence processes of various medium-sized fruits was applied and tested (Figure 1).

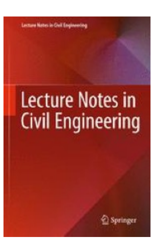


Figure 1. Particular of the prototype by the Department of Agricultural and Environmental Sciences (DiSAA) of the Università degli Studi di Milano and an example of data acquisition on apple.

Table 1. Wavelengths of the three sensors chosen for the prototype.

Wavelengths (nm)						
Sensor 1	410	435	460	485	510	535
Sensor 2	560	585	645	705	900	940
Sensor 3	610	680	730	760	810	860

Currently in its prototype phase, the device represents a simplified version compared to comprehensive commercial instruments. It operates within a limited spectral range, using 18 wavelengths ranging from 410 to 940 nanometers, covering both the visible and a segment of the Near-Infrared range (Table 1). The prototype was built at the Department of Agricultural and Environmental Sciences (DiSAA) of the Università degli Studi di Milano.

The sensing hardware is a commercial chipset and consists of three sensor devices for spectral acquisition in a range from visible to NIR. Every of the three sensor devices has 6 independent on-device optical filters whose spectral response is defined in a range from 410 nm to 940 nm with FWHM of 20 nm (AS7265x, SparkFun Electronics®, Niwot, USA). Each sensing device has two integrated LEDs (white and red). Other components of the prototype were fabricated internally by the DISAA, through additive manufacturing using a 3D printer.

The prototype was tested on samples of Fuji apples (Malus domestica, Fuji) - and Passacrassana pears (Pyrus communis, Passe Crassane), Dottato figs (Ficus carica L.), kiwis (Actinidia deliciosa) (data not shown, ongoing dataset acquisition) - to evaluate the entire ripening progression from early stages to decay. The study also aimed to validate the effectiveness of this technique and the prototype on matrices with different exocarp characteristics.

Data acquisition was conducted on different sampling days to understand variations in optical and qualitative parameters. As the prototype is still under development, a commercial instrument was used as a reference instrumental benchmark: a benchtop instrument operating in the range of 380 to 1650 nanometers (Corona® Process, ZEISS, Germany).

To analyze the acquired optical data and extract high-quality information, several chemometric techniques could be employed. These techniques are essential when dealing with intrinsically complex spectroscopic data. The data were analyzed in the MATLAB environment, version 2023b (Math-Works, Inc., Natick, MA, USA) using the PLS-Toolbox package (Eigenvector Research, Inc., Manson, WA, USA).

After an initial visual exploration of the spectra, the unsupervised technique PCA (Principal Component Analysis) was applied to spectroscopic data from the prototype. This statistical technique is used to reduce the complexity of intricate data matrices while preserving as much variability as possible and simultaneously providing qualitative information about the analyzed samples. To verify the performance of the pre-prototype, it is essential to use this type of technique to first ensure that the device can distinguish samples at different levels of maturity.

3. Results and discussion

Figure 2 shows, for the apple samples, the optical outputs from the prototype and the spectra from the commercial spectrophotometer.

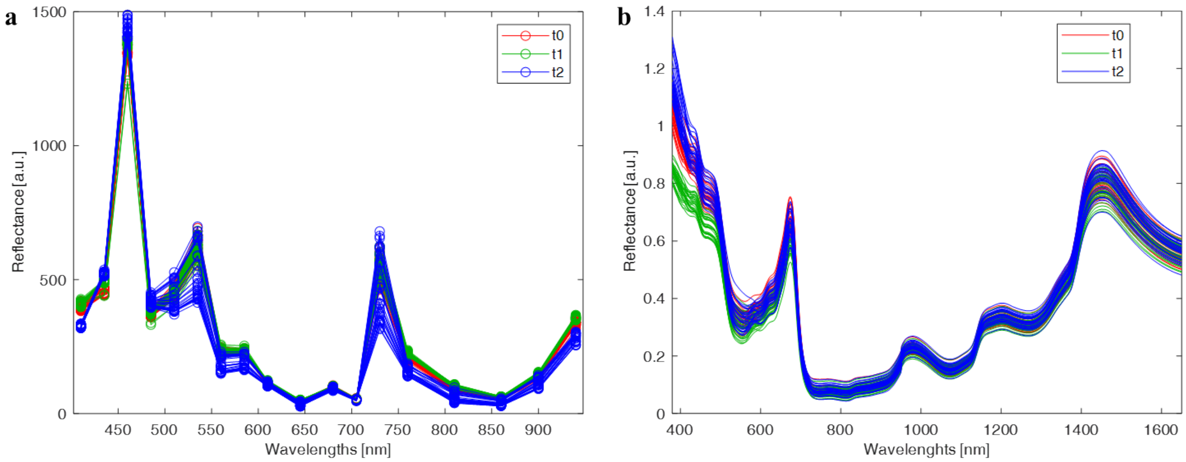
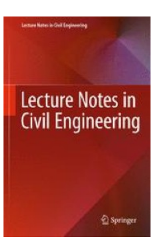


Figure 2. Apples dataset. Optical outputs from the prototype (a) and spectral data set from the commercial spectrophotometer (b).

A discrete trend of the optical outputs for the prototype can be seen due to the set of values measured at the 18 wavelengths of the 3 sensors on board the system. Otherwise, the commercial spectrophotometer acquires continuous spectra in reflectance mode in the vis/NIR spectral range. In Figure 3 (a), an example Score Plot (PC1 vs PC2) of apple samples is presented, where the distribution of the samples can be observed. In Figure 3 (b), the Loadings are shown, representing the contribution of the original variables, in this case, wavelengths, to the components.

From Figure 3 (a), two clusters can be clearly distinguished: the t0 and t1 samples were acquired a few days apart, while the t2 samples were analyzed after almost a month of maturation. Despite the minimal visible changes over the days, the device was able to clearly distinguish the t2 samples, showing that along PC1, we can observe the progression of senescence.



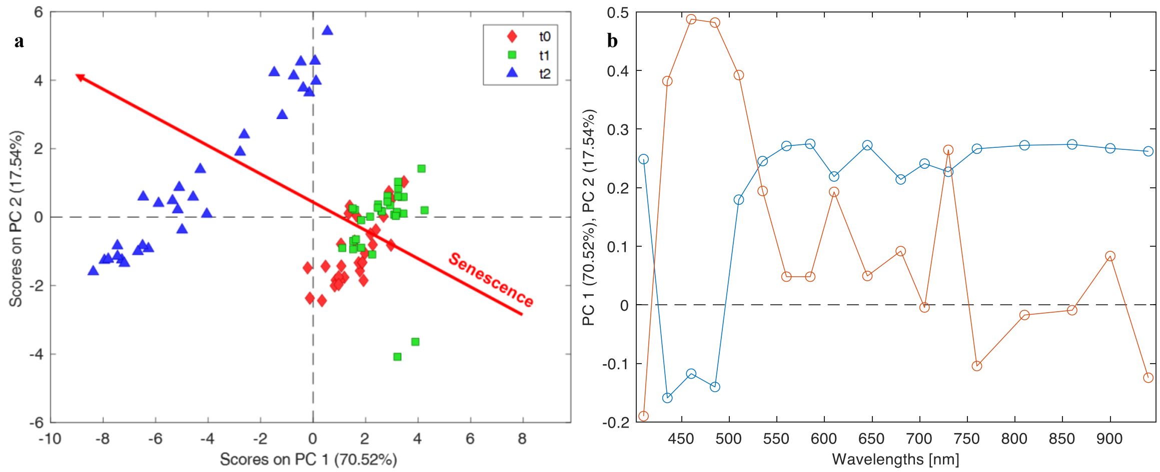


Figure 3. Score plot (a) and loadings plot (b) of the PCA results for apples from the pre-prototype.

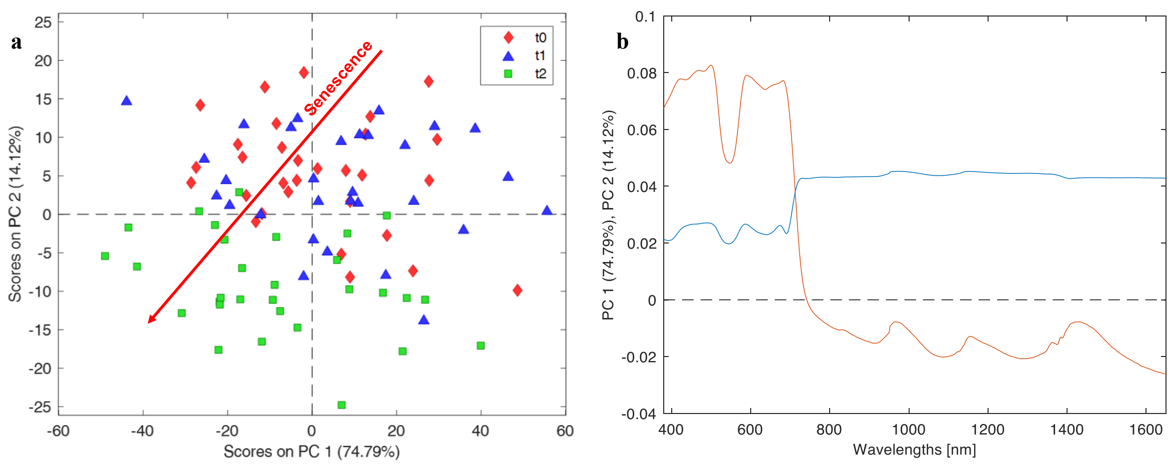
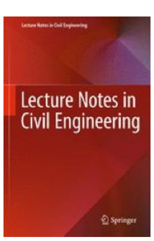


Figure 4. Score plot (a) and loadings plot (b) of the PCA results for apples from the commercial spectrophotometer.

As a comparison and benchmark, the same data analysis procedure was applied to the spectral data obtained with the commercial instrument. The results of the PCA applied on optical data deriving from commercial device (PC1, 74.79% vs. PC2, 14.12%) are shown in Figure 4. The Score Plot of the apple samples is shown in Figure 4(a), allowing the distribution of the samples to be seen. The loadings, or the contribution of the original variables (wavelengths) to the components, are shown in Figure 4(b).

The PCA shows a similar trend with a similar ability to discriminate the samples of different aging times compared to what was obtained with the data from the prototype.



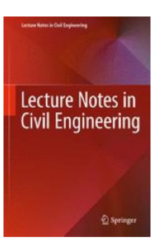
The results are very preliminary and will need to be validated with additional experimental campaigns. Furthermore, to achieve a more comprehensive assessment of the prototype's performance, it will be important to develop quantitative predictive models for the key quality parameters of various types of medium-sized fruits. This will also allow for the evaluation of potential improvements to the prototype hardware by identifying and implementing necessary technical modifications.

4. Conclusions and future perspectives

The very preliminary results from this research could be considered promising, highlighting the potential for implementing real-time quality monitoring of these fruits. Spectroscopy, with its numerous benefits, is already widely applied in the agri-food sector. However, the true novelty of this project lies in the planned enhancement of the prototype. This proposed upgrade aims to equip operators with the ability to supervise batches, accurately classify the products, and effectively inform the end consumers.

Implementation of these devices has the potential to revolutionize the management of harvesting and storage processes, thereby optimizing the quality of agricultural products for consumption. The envisioned role of the prototype represents a major step forward in improving operational efficiency within the agricultural sector.

Further experimentation and structural modifications of the prototype will certainly be necessary to make it usable completely independently and even by non-specialized operators. The next steps will be to increase the quantity and variety of samples, as well as to advance the Technology Readiness Level (TRL) of the prototype which now corresponds to a number 3 (Analytical and experimental critical function and/or characteristic proof-of-concept) on the scale of 9 (System proven in operational environment).



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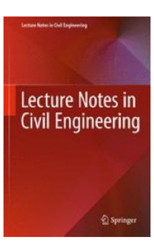

5. CONCLUSIONS AND FUTURE PERSPECTIVES

The use of vis/NIR spectroscopy in agri-food applications has demonstrated significant potential across multiple experiments described in this PhD thesis. These studies highlight the increasing interest in small-sized, cost-effective, and user-friendly portable devices capable of rapid and non-destructive analysis of quality parameters throughout various stages of the supply chain. Several prototypes were developed and tested, showing promising results for monitoring quality traits in agricultural products, such as moisture content and soluble solids in tomatoes, as well as sugar and phenolic content in grapes. However, further research is needed to enhance the robustness of predictive models through larger datasets and improved optical configurations. Particularly with two of the three devices, it will be essential to conduct large-scale trials and involve companies in testing these technologies to expand the range of matrices used and increase the volume of data. Technologies are advancing rapidly, and soon it will be possible to acquire various types of sensors to further enhance prototype performance. However, these prototypes will need to be re-tested with the new configurations.

One of the key strengths identified was the potential to develop a low-cost, easily adaptable device for field use, which could support small-scale farmers and producers in determining optimal harvest times and ensuring quality management during post-harvest. The application of such devices could be also extended to other small fruits and matrices, broadening their usability and impact. The miniaturization and integration of prediction algorithms into the device software were suggested to enable real-time visualization of the parameters, promoting ease of use for non-expert personnel.

In the context of sustainability, vis/NIR spectroscopy has been established as a green technology that significantly reduces the environmental impact compared to conventional wet-chemical analyses. This thesis explored and quantified the environmental benefits of adopting portable optical solutions with an LCA study, highlighting their suitability for minimizing chemical usage and promoting sustainable practices. Furthermore, the potential for developing interconnected optical systems and remote storage of databases was discussed as a mean to improve prediction accuracy and support a network-based approach for quality monitoring. While benchtop instruments offer high precision, their environmental footprint and complexity make them less suitable for widespread field deployment. The simplified prototypes, though initially less precise, proved to be a more sustainable option, especially when normalized for performance. This finding underscores the need to balance analytical performance with environmental considerations in the development of future optical devices.

The experimentation involving the automated system for fermentation management emphasizes the potential of automated systems in enhancing efficiency and sustainability across different sectors of the agri-food industry and that could potentially be implemented with optical instruments in the future. The SWOT analysis of the automated fermentation system highlights its strengths in large-scale wineries, where the benefits of automation are most pronounced. Automation allows for optimized lean production, reducing resource consumption and minimizing human error, which results in improved product quality and worker conditions. Moreover, the ability to monitor fermentation in real-time and remotely leads to better process control compared to traditional methods. In larger wineries, the reduced payback period makes automation a highly viable investment, despite the higher costs of more sustainable nutrient inputs.

However, smaller wineries, particularly those managed by older generations, may face challenges in adopting such technologies due to the initial financial burden and strong ties to traditional methods. In this context, the transition towards automation aligns with the broader trend of technological modernization and environmental consciousness within the wine industry, but the scale of production remains a critical factor in determining feasibility. Despite these barriers, automation is positioned as an essential tool for meeting Industry 4.0 requirements and achieving more sustainable production practices in larger wineries.

Research regarding the determination of acrylamide, on the other hand, has demonstrated the versatility of this technology beyond the viticulture sector. The promising results from the predictive models suggest that vis/NIR spectroscopy could become a rapid, non-destructive method for estimating acrylamide levels. To strengthen these findings, further validation with larger sample sets and diverse acrylamide concentrations will be essential. The study deliberately focused on a challenging sample type, cocoa-based biscuits with a dark color, to assess the instrument's ability to detect acrylamide, a compound closely linked to the Maillard reaction. Given the potential introduction of regulatory limits for acrylamide content, the implementation of vis/NIR devices in-line within production processes would provide a significant advantage. This technology could enable real-time monitoring of product compliance, enhancing food safety and reducing waste. Additionally, developing classification models for acrylamide would allow manufacturers to swiftly identify non-compliant samples before legal limits are enforced. The potential applications of this technology could extend to other food matrices, reinforcing the role of optical analyses in modernizing quality control practices across the agri-food industry.

Both automated systems in wineries and vis/NIR spectroscopy in food safety represent significant steps toward smarter, more sustainable production. These technologies offer solutions that reduce environmental impact, streamline operations, and enhance product quality, aligning with the principles of Industry 4.0 and a growing emphasis on sustainability in agriculture and food processing. Further research and development will be crucial in refining these tools and broadening their application to various sectors within the industry. Other production processes will need to be identified where automated controls can be synergistically utilized with different types of sensors, enabling testing on various matrices and continuously improving the performance of these tools and technologies.

Vis/NIR spectroscopy showed great promise for supporting precision agriculture and enabling more sustainable practices in the agri-food sector. The integration of these technologies into the supply chain, from field to processing phases, can streamline quality control processes and optimize production efficiency, contributing to the broader goals of agriculture 4.0. Future research should focus on refining these prototypes, expanding their applications to other crops, and exploring the possibilities for integrating such devices into smart agricultural systems to achieve a comprehensive, data-driven approach to quality management.

The experimentations and technologies discussed in this thesis can significantly contribute to achieving SDG 12 of the 2050 Agenda, which focuses on responsible consumption and production. Automated systems in the winemaking process optimize resource usage by reducing waste, improving energy efficiency, and minimizing human error. By enabling precise, real-time control over fermentation, these systems enhance product quality while minimizing environmental impact. This aligns with SDG 12's goal to reduce resource consumption and ensure sustainable production processes.

Vis/NIR spectroscopy, particularly in detecting acrylamide in food products, offers a non-destructive, rapid method to ensure food safety without generating chemical waste. Its ability to monitor multiple quality parameters with a single device streamlines quality control, reduces waste, and promotes more efficient use of resources across food supply chains. Moreover, this optical technology can be applied to various food matrices, making it versatile for broader applications, supporting sustainable practices and reducing food loss.

Both technologies embody innovation that advances sustainable industrial practices while promoting environmentally friendly solutions. Their implementation across different sectors can lead to a significant reduction in environmental impact, aligning with SDG 12’s objective to achieve sustainable consumption and production patterns by 2050.

With regard to the three prototypes developed, the first two reached a Technology Readiness Level (TRL) of 4 out of 9, while the third stands at a TRL of 3. Further development and testing will be required to enhance the applicability of these prototypes in the agri-food sector.
Nevertheless, these advancements align perfectly with the goals of Agenda 2050, which, as previously mentioned, aims to promote innovative and sustainable technologies to address global challenges related to food security and resource efficiency. Continued investment in the research and development of tools like vis/NIR spectroscopy will not only contribute to the transition toward more resilient and sustainable food production systems but will also support efforts towards a low-carbon future, in line with long-term sustainability objectives.

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7. APPENDICES

Awards

1. Winner of the SISNIR grant offered by Società Italiana di Spettroscopia NIR “Bando di Formazione 2021-2023” to participate in a training course of recognized value.
2. Winner of the SISNIR grant offered by Società Italiana di Spettroscopia NIR to attend the Conference NIR Italia 2022.
3. Winner of the SISNIR grant offered by Società Italiana di Spettroscopia NIR to attend the 21st International Conference on Near Infrared Spectroscopy (NIR2023).

Conferences attendance

1. **The 20th International Conference on NIR** (online) – poster


NIR2021
18-21 October
www.nir2021.com
Sense The Real Change

Certificate of Attendance

This is to certify that

Alessia Pampuri

attended the NIR2021 International Conference
held as an on-line meeting in Beijing, China,
18-21 October 2021
with poster presentation entitled
"Smart-HAND: a simplified LED device for intact olives quality evaluation"

NIR2021 Chairman

Prof. Hongfu Yuan
Beijing University of Chemical Technology
Yuan Hongfu

2021 Beijing

2. **NIR Italia 2022 – National Symposium of NIR Spectroscopy** – oral presentation


NIR Italia 2022 7-9 June 2022
beyond spectral range

CERTIFICATE

This is to certify that

Alessia Pampuri

presented in the NIR Italia 2022 Conference
that was held at InnoRenew CoE,
7-9.06.2022, Izola, Slovenia

Organizing Committee
Anna Sandak

Conferences attendance

3. **FRUTIC 2022** - poster

Valencia (Spain) • 29th June - 01st July

2022 Frutic 14th International Symposium

CERTIFICATE ATTENDANCE

This certificate awarded to

ALESSIA PAMPURI

for attending the 14th International FRUTIC Symposium
June 29 – July 1 2022, at Valencia, Spain.

Valencia, 1st July 2022

This activity was awarded by:

José Blasco
Chair of FRUTIC 2022

I virtual Workshop on the Developments in the Italian PhD
Research on Food Science, Technology and Biotechnology

Asti, September 19th-21nd, 2022

Certificate of
Successful Learning Outcomes

PhD student PAMPURI Alessia

I year of the PhD Course in
Food Systems (University of Milan, XXXVII cycle)
has participated to the plenary lecture and
has acquired n° **2** credits (**CFU**) with
a grade **A** (score 93 & 87 out of 100)

Head of PhD Course in Food Systems
(Prof. Diego Mora)

Italian Network of the PhD Courses in Food Science Technology & Biotechnology

4. **26th Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology** – miniposter

5. **The 21st International Conference on NIR** – poster


NIR2023 INNSBRUCK NIR 2023
21ST INTERNATIONAL CONFERENCE ON NEAR INFRARED SPECTROSCOPY
GOOD VIBRATIONS, SMOOTH CONTOURS
August 20-24,2023 Austria
NIR2023.at

CERTIFICATE OF ATTENDANCE

This is to certify that

Alessia Pampuri

has attended the

21st INTERNATIONAL CONFERENCE ON NEAR INFRARED SPECTROSCOPY

held from
20 - 24 August 2023

at
Congress Innsbruck, Austria

Christian W. Huck   Wolfgang Lindner


CERTIFICATE OF ATTENDANCE

This to certify that
ALESSIA PAMPURI
Università degli Studi di Milano
attended the
27TH WORKSHOP ON THE DEVELOPMENTS IN THE ITALIAN PHD RESEARCH ON FOOD SCIENCE TECHNOLOGY AND BIOTECHNOLOGY

held on September 13th-15th, 2023
at the Department of Agricultural Science,
University of Naples- Federico II, Portici (NA), Italy

The Local Organizing Committee

6. **27th Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology** – poster

7. **28th Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology** – oral presentation

Uni ct AGRICOLTURA, ALIMENTAZIONE E AMBIENTE

28° Workshop on the Developments in the Italian PhD research on Food Science, Technology and Biotechnology
Catania, 18-20 September 2024

GSICA SISS SISTAL SIMTREA

Certificate of Participation

Delivered to

Alessia Pampuri

Conference Convenor
Prof. Biagio Fallico

1. **School of Multivariate Analysis** – DiFAR, University of Genoa

Università di Genova | **DIFAR** DIPARTIMENTO DI FARMACIA

Sezione di Chimica e Tecnologie Farmaceutiche e Alimentari
Gruppo di Ricerca di Chimica Analitica e Chemiometria
Viale Cembrano 4 – 16148 GENOVA

Con la presente si attesta che **Alessia Pampuri**, Università degli Studi di Milano, ha partecipato alla SCUOLA DI ANALISI MULTIVARIATA, Microsoft Teams, 17-21 Gennaio 2022, per un totale di 30 ore di lezione.

Riccardo Leardi

Prof. Riccardo Leardi

UNIVERSITÀ degli STUDI di GENOVA – DIPARTIMENTO DI FARMACIA

Genova, 21/01/2022

2. **School of Scientific Computing with MATLAB 2023** – Basic Course

SCUOLA DI CALCOLO SCIENTIFICO CON MATLAB® Edizione XV

Attestato di partecipazione

Scuola di Calcolo Scientifico con MATLAB 2023

CORSO BASE
17 - 21 Luglio 2023

PROGRAMMAZIONE E CALCOLO SCIENTIFICO CON MATLAB | 32 ore

PAMPURI ALESSIA

U4learn DiFC

3. **Parma Summer School 2023** – Innovative Food Products

PARMA SUMMER SCHOOL

26 – 28 SEPTEMBER 2023

CERTIFICATE OF ATTENDANCE

Awarded to

Alessia Pampuri

for participating in the Parma Summer School 2023:

"Innovative Food Products"

The Scientific Committee

events@efsa.europa.eu

efsa EUROPEAN FOOD SAFETY AUTHORITY

UNIVERSITÀ DI PARMA SCUOLA DI STUDI SUPERIORI IN ALIMENTI E NUTRIZIONE

UNIVERSITÀ DI PARMA

UNIVERSITÀ CATTOLICA del Sacro Cuore

Internship abroad

Research period at Stellenbosch University (Stellenbosch, South Africa) from 11th April 2024 to 9th September 2024

Tutor: Professor Marena Manley

Stellenbosch UNIVERSITY IYUNIVESITHI UNIVERSITEIT

AGRISCIENCES
EYENZULULWAZI NGEZOLIMO
AGRIWETENSKAPPE

Department of Food Science
ISebe leNzululwazi yoKutya
Departement Voedselwetenskap

20th February 2024

To Whom It May Concern

I, the undersigned, Marena Manley, Professor at the Department of Food Science at Stellenbosch University (Stellenbosch, South Africa) certify that during the period 1 April 2024 to 30 September 2024, Ms Alessia Pampuri (PhD student in Food Systems at the University of Milan, Italy) will conduct research with me and my research group for five months.

Ms Pampuri will not receive any financial compensation from me and will cover her own travel, accommodation and maintenance expenses for the entire duration of the research period.

Please feel free to contact me by email or mobile phone if you have any questions about her visit.

Yours sincerely

Prof Marena Manley

Stellenbosch University
Department of Food Science
Email: mman@sun.ac.za
Mobile: +27 82 771 5716

Credits and soft skills

Mandatory specialized-teaching courses attended:

1. **E-sensing technologies and chemometrics**

   Professor: Alamprese C.

   Date: February 2022

   Food Systems course: Yes

   CFU: 5

2. **Sustainability concepts in food technology - methodological approaches and case studies**

   Professors: Farris S., Fracassetti D., Laureati M., Lavelli V., Limbo S., Marti A., Pagliarini A.

   Date: March 2022

   Food Systems course: Yes

   CFU: 4

3. **Industry 4.0 for the agro-food system**

   Professors: Guidetti R., Beghi R., Giovenzana V.

   Date: March 2022

   Food Systems course: Yes

   CFU: 3

Mandatory soft skills-courses attended:

- Academic writing - part I: 26/01/2022, 2 hours
- Academic writing - part II: 09/02/2022, 2 hours
- Language coaching, interpersonal skills - part I: 14/03/2022, 2 hours
- Language coaching, interpersonal skills - part II: 17/03/2022, 2 hours
- Basic preparatory lesson on IP and patents: 22/03/2022, 2 hours
- Open science, Scientific Area - part I: 01/04/2022, 2 hours
- Open science, Scientific Area - part II: 08/04/2022, 2 hours
- Grantmanship - part I (first part): 19/05/2022, 2 hours
- Grantmanship - part I (second part): 20/05/2022, 2 hours
- Grantmanship - part II (first part): 21/06/2022, 2 hours

- Grantmanship - part II (second part): 23/06/2022, 2 hours
- Communication and new media - part I: 28/06/2022, 2 hours
- Research evaluation - English edition: 12/09/2022, 3 hours
- Introduction to research valorization: 27/09/2022, 2 hours
- Introduction to Open Science and Research Integrity (4EU+ seminar): 22/02/2023, 1.5 hours
- Open Access and Open licenses (4EU+ seminar): 24/03/2023, 1.5 hours
- Publishing with the rights retention strategy: is it for me? (4EU+ seminar): 31/03/2023, 1.5 hours
- Preprint and open peer review (4EU+ seminar): 11/04/2023, 1.5 hours
- Enhancing value by creating business: spin-offs at University of Milan - Part I (eng): 17/04/2023, 3 hours
- Enhancing value by creating business: spin-offs at University of Milan - Part II (eng): 18/04/2023, 3 hours
- Predatory publishers and identifying fraud - how to identify dubious providers? (4EU+ seminar): 27/04/2023, 1.5 hours
- Language coaching - Interpersonal Skills: Interview Skills - part I: 22/05/2023, 2 hours
- Language coaching - Interpersonal Skills: Interview Skills - part II: 23/05/2023, 2 hours
- The FAIR principles in science, technology, and engineering – How to write a good Data
- Management Plan for FAIR research data (4EU+ seminar): 26/06/2023, 3 hours
- Self Branding: Competencies and employability of PhD holders: 29/06/2023, 3 hours
- Researcher ID and digital strategy: creating a real ORCID ID to be visible (4EU+ seminar): 03/07/2023, 1.5 hours
- Open research software - how to disseminate it? (4EU+ seminar): 06/07/2023, 1.5 hours
- Research Integrity I: Online (January 2023), 6 hours
- Research Integrity II: 21/02/2023, 2 hours
- Searching for open access works (4EU+ seminar): 11/05/2023, 1.5 hours
- Workshop on producing scientific content for new media: 05/07/2023, 3 hours
- Job seeking with a PhD in hand - Part 1: 16/02/2024, 2 hours
- Job seeking with a PhD in hand - Part 2: 21/02/2024, 2 hours
- Public speaking - First meeting: 19/02/2024, 2 hours
- Public speaking - Second meeting: 20/02/2024, 2 hours
- Advanced lesson on the use of IP for innovation - Part 1: 20/02/2024, 1 hour

- Advanced lesson on the use of IP for innovation - Part 2: 21/02/2024, 2 hours
- Preparation of a dissemination plan for a research project: 19/02/2024, 2 hours
- Teaching and learning I: 28/02/2024, 4 hours
- Teaching and learning II: 28/02/2024, 5 hours
- Evaluation of integrated sustainability for global health: 28/02/2024, 5 hours

Total hours attended first year: **29**

Total hours attended second year: **39**

Total hours attended third year: **27**

Total soft-skills hours: **95**