Optical analysis based on near-infrared spectroscopy for nutrition monitoring, food scanning and industrial applications

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ABSTRACT

New food analysis enabling approaches offer significant advantages over traditional methods in terms of test speed, control, low cost, ease-of-operation, and data management, and require minimal equipment and user involvement. Among them, near infrared spectroscopy has a great potential for providing a user-friendly, scanning on-the-go scenario. It indeed allows peculiar rapid multi-component non-invasive and non-destructive analysis, and is a greener alternative to reagent-based analytical technique; no need for reagents and limited waste after the analysis. All these advantages, together with a progressive reduction of system cost and size, make it a very promising methodology in terms of range of applicability, with high scientific and commercial impact. However, being a nonspecific method, it must be associated with complex multivariate statistical and chemometric tools for spectral dataset analysis to extract relevant chemical information. Indeed, calibration and tuning of the systems are performed according to the desired application. Moreover, in a view to simplify apparatus and decrease cost, critical design criteria are represented by the proper selections of the wavelength range for source and detector, and of the measurement setup. In this work we describe a multi-platform optimized analytical method based on the most informative wavelength range in the NIR region for quantitative measurement of sugar content in aqueous solutions and beverages by means of absorbance spectroscopy. The achieved results were then profitably exploited as a cue for design and as a reference for comparison to develop a simplified LED-based optical platform for sugar content measurement in beverages. The proposed analytical and sensor solution was conceived for tracking dietary sugar intake in liquid foods, as e-health technological answer for the serious and widespread problem of unwitting over-intake of sugar, far above the recommended assumption, and also as a possible diet companion platform for dietary control of diabetes patients.

Contents

LIST OF PUBLICATIONS

Rateni, G., Dario, P., & Cavallo, F. (2017). Smartphone-based food diagnostic technologies: a review. *Sensors*, *17*(6), 1453.

Bonaccorsi, M., Rateni, G., Cavallo, F., & Dario, P. (2017, October). In-line industrial contaminants discrimination for the packaging sorting based on near-infrared reflectance spectroscopy: A proof of concept. In *SENSORS, 2017 IEEE* (pp. 1-3). IEEE.

Rateni, G., Francini, A., Sebastiani, L., Dario, P., & Cavallo, F. Multi-platform sugar content measurement in acqueous solutions and beverages from optimized NIR spectral analysis
Submitted on June 20, 2018 in Analytical Methods, Royal Society of Chemistry.

Rateni, G., Giardini, M., Francini, A., Sebastiani, L., Dario, P., & Cavallo, F. Design, development and testing of a portable LED-based optical platform for sugar intake tracking in beverages
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I. INTRODUCTION

In this chapter an introductory overview of analytical devices able to enhance the ordinary smartphone with food diagnostic capabilities is provided. The latest relevant research works are illustrated, categorized according to the diagnostic method, with each working principle explained. Furthermore, recent commercial exploitations in this sector are described, showing the most interesting solutions. Finally, perspectives on the current limitations, challenges, and future directions of this novel, emerging field of research are discussed.

A. Smartphone-based diagnostic platforms for food analysis

Mobile diagnostics is gaining more and more attention in healthcare, environmental monitoring, and agro-food sectors, allowing rapid and on-site analysis for preliminary and meaningful information extraction. The aim is to bypass the use of expensive and bulky instrumentation-based routine tests, performed by trained personnel, with the goal of cost saving and time efficiency. Not by chance, recently a Horizon Prize regarding the challenge of developing a rapid non-invasive food scanning device has been launched [1], reflecting the fact that it comes to a hot topic not only in research, but also for the market. Moreover, this approach could have a substantial positive effect on health, environmental, and food diagnostics technologies in both developed and developing countries, leading to a democratization in measurement science, thanks to the massive volume of mobile phone users spread globally [2]. Indeed, the ubiquitous availability of smartphones throughout the world enables a broad accessibility. Smartphones can be deployed in a variety of environments, including remote or underdeveloped rural regions. For example, the current methods for ensuring food safety rely on routine, but highly resource-intensive laboratory-based examination of chemicals and/or foodborne pathogens. In remote areas, where resources are scarce, sending specimens to an analysis laboratory can be difficult. Smartphone-based analytical platform, instead, could bypass these logistic issues via on-site testing or remote confirmation of detection [3].

Smartphones are equipped with numerous components that can be employed for measurement and detection, such as a fast multicore processor, digital camera, battery, visual display, and intuitive user interface. Smartphones also possess several wireless data transfer modalities (e.g., cellular data service, Wi-Fi, Bluetooth), allowing test results to be displayed immediately to the user and/or transmitted to cloud databases. Nevertheless, smartphones can-not function alone as laboratory instruments. Rather, they need to be augmented by other accessories. Such augmented devices have great potential as mobile diagnostic platforms for food analysis. In recent years, many external sensor modules have been designed and integrated with smartphones to extend their capabilities for extracting more-sophisticated diagnostic information. These portable, low-cost devices have the potential to run routine tests, which are currently performed by trained personnel using laboratory instrumentation, rapidly and on-site, thanks to the global widespread use of cellphones. In the field of food, this ability translates to improved awareness of what we eat. Examples include empowering the mobile phone of an allergic subject with personalized diagnostic capability, or allowing rapid inspection in the case of suspected contamination by foodborne pathogens or other hazardous contaminants. Moreover, even the agro-food sector could benefit from the development of portable lab-on-smartphone platforms, allowing on-field extraction of valuable data about a crop’s maturity and health state. With advances in micro-manufacture, sensor technology, and miniaturized electronics, diagnostic devices on smartphones will be used increasingly to perform biochemical detections in healthcare diagnosis, environmental monitoring, and food evaluation in the near future [4].

Much effort has been directed toward using the mobile phone as a sensing device, as described in Li et al. [5]. The increasing number of measurement applications on smartphones is due to their growing capabilities of sensing physical quantities by leveraging new embedded sensors or through wireless and wired connection possibilities and smart visual interfaces, to receive measurements from different external systems [6]. While many works have focused on the development of point-of-care systems for biomedical purposes as reported in Xu et al. [7], smartphone diagnostic platforms also have emerged in other important applications. Recent existing reviews by Roda et al. [8], Liu et al. [9], and Yang et al. [10] have described these advancements.

Smartphone-based food diagnostic approaches can be divided into two categories: lab-on-smartphone biosensors and smartphone optical and spectroscopy. Studies are presented in chronological order for each detection method. An explanation is provided of the technique employed and the added value the integration of a smartphone in the loop provides to the approach.

1) *Lab-On-Smartphone Biosensors*

Biosensors are analytical devices that integrate a bio-receptors with an appropriate transducing method to detect analytes of interest. The specific interaction between the target analyte and the receptor produces an output measurable signal, which highlights the presence of the sought element. Thus, this approach has high selectivity, since direct detection of the target analyte is achieved. However it always includes an invasive sample pre-treatment phase to give rise to transduction. Biosensors allow low-cost, and fast analysis, with results in a few minutes, and show perspectives for miniaturization and portability. Taking advantage of the combined use of smartphone and adapted biochemical assay, biosensor-based analytical systems are promising tools for on-site detection of analytes including contaminants, drugs, pesticide residues, and foodborne pathogens. A detailed overview of the revised Lab-On-Smartphone Biosensors works is provided with Table 1.

Table 1. Summary of recent lab-on-smartphone biosensor platforms.

Detection Target	Methodology	Materials	LoD/Test Time/Performance	Smartphone Use	Ref.
Escherichia coli in water	Fluorescent imaging	Antibody, quantum dots, UV LED	5–10 CFU mL^{-1}	Cellphone imaging with camera attachment	[11]
rbST antibodies in	Microsphere	Antibody, quantum dots,	80% true-positive rate	Cellphone	[12]

milk	fluorescent immunoassay	UV LED and white LED	and 95% true-negative rate	imaging with camera attachment	
Lactose and galactose in undiluted food samples	Engineered bacteria fluorescence	Blue light and optical filter	1–1000 mM	Cellphone imaging	[13]
Peanut allergen in food samples	Colorimetric assays	ELISA allergen test kit, cellphone attachment with 2 test tubes and 2 LEDs	~1 parts per million (ppm), 20-min preparation phase	Cellphone assay with camera attachment	[14]
Aflatoxin B1 in maize	Lateral flow immunoassay	Paper strip, close-up lens and a white LED	5 μg/kg	Smartphone imaging via LFIA reader adapter	[15]
BDE-47 in food sample	Microfluidics and competitive ELISA	Arduino Nano, PCB, microfluidic chip	Readout time of 15 min and input sample volume considerably reduced	Smartphone as power source, imaging reader and cloud sender	[16]
Red wine properties	Paper microfluidics, colorimetric assay, and PCA	Chemical dyes	Successful distinction of red wines by their grape varieties and oxidation.	Smartphone imaging	[17]
Amines as indication of foodborne pathogens in meat	Membrane technology colorimetry and unsupervised chemometric tools	Dyes	Down to 1 ppm concentration of amine	Camera imaging	[18]
Antibiotic residues in milk	SPE and fluorescence spectroscopy	Photography lightbox with fluorescent light	LoC 0.50 mL^{-1} and LoQ 1.50 μg mL^{-1}	Smartphone camera used as spectrometer	[19]
Glutamate in food compound, instant soup and wines	Paper-based colorimetric assay	Glutamate-specific enzyme	0.028 mmol L^{-1}	Camera acquisition and analysis	[20]
ALP as indicator of incorrect milk pasteurization	Disposable lateral flow-through strip	Sample pad	0.1 U L^{-1}, within 10 min with a detection range of 0.1–150 U L^{-1}	Image acquisition and Matlab analysis	[21]

Table 1. *Cont.*

OA and STX in shellfish	Competitive immunoassay strip	3D-printed smartphone strip adapter	2.800 ng mL^{-1} for OA and 9.808 ng mL^{-1} for STX in 30 min	Camera acquisition via strip adapter and data processing	[22]
Fluoride in water	Colorimetric imaging	Compact sample chamber adapter for smartphone	Linear range 0–2 mg L^{-1}	Smartphone colorimeter	[23]
Catechols in water	Colorimetric imaging	96-well sensor array, light-tight box, white LED	PCA, HCA and LDA for quality discrimination and PLS for quantitative determination	Smartphone colorimeter coupled to remote server	[24]
V. parahaemolyticus in fish samples	Colorimetric immunoassay	Biosensor cartridge, lens-free CMOS image sensor, Wi-Fi module	1.4 × 10^4 CFU mL^{-1}	Dedicated app to operate the system and upload on internet server	[25]
Escherichia coli O157:H7 and *Salmonella enterica*	DNA transduction on microfluidic device	Magnetic beads	Down to 20 genomic copies of *E. coli*	Custom written app for cell phone image analysis	[26]
Clenbuterol	Electric field-driven immunoreaction	Functionalized electrodes	0.076 ng mL^{-1} CLB in 6 min	USB Smartphone tool biochip	[27]
Pattern recognition of Brazilian honey samples	Cyclic voltammetry assay	Electrode of gold, homemade potentiostat with USB connection and Bluetooth module	Successfully generation of voltammetric fingerprints of numerous honey samples	Chemometric data processing on smartphone	[28]

Fluorescence Imaging Using Smartphone

Fluorescence imaging is the visualization of fluorescent dyes as labels for biological or chemical molecules of interest. It enables a wide range of experimental observations including the location of gene expression, protein expression and molecular interactions in cells and tissues. To label a biological molecule of interest, a fluorescent marker, which is able to bind the target molecule, has to be introduced. The setups found in the revised works, which perform fluorescence imaging, also include a monochromatic light source, typically a UV LED, to generate the dye excitation and a smartphone camera used as detector to collect and measure the fluorescence intensity. The presented systems, proposed for a specific detection application, have the advantage of being applicable to other targets of interest through the use of different specific molecular dyes.

Zhu et al. [11] developed a portable Escherichia coli detection platform for screening of water and food samples. The cellphone-based fluorescence imaging platform was specifically realized to quantify the bacterial concentration in a water sample loaded into glass capillaries, opportunely functionalized with antibody directed against E. coli. Secondary antibodies, conjugated with quantum dots, were subsequently dispensed into the capillaries and served as the fluorescence signal. Quantum dots are inorganic nanocrystals with unique optical and chemical properties that give them exceptional brightness and photo-stability. In this case, UV-LEDs provided the excitation signal. The emission from the quantum dots through an additional lens was conveyed to the phone camera unit. A light-weight (~28 g) and compact (3.5 cm × 5.5 cm × 2.4 cm) attachment to the existing camera unit of a cellphone was designed to host the diagnostic platform. By quantifying the fluorescent light emission from each capillary tube, the concentration of E. coli in the sample was determined. The authors reported that the test can be completed within 2 h, including sample preparation, sample loading and incubation, with a detection limit of 5–10 CFU mL−1 in buffer solution. They also demonstrated the efficacy of this approach using fat-free milk as a matrix, where a similar detection limit was achieved. The same concept was used by Ludwig et al. [12] for the detection of the presence of anti-recombinant bovine somatotropin (rbST) antibodies in milk, which are endogenously produced upon administration of rbST, a milk production enhancer in dairy cattle which is illegal in the EU and represents a public health concern in the US. To monitor the presence of this biomarker, multiple immunoassay microspheres were used for the detection of multiple antibodies simultaneously within a small volume of a single sample. In addition to the same setup previously described, a white LED for dark-field imaging of all microspheres present in the sample was used. The cellphone-based diagnostic platform was successfully applied to milk sample extracts from rbST-treated and untreated cows. An 80% true-positive rate and 95% true-negative rate were achieved. The designed system can be adapted to any available cellphone that has a camera module simply by modifying the dimensions of the cellphone holder and 3D-printing another one accordingly (Figure 2).

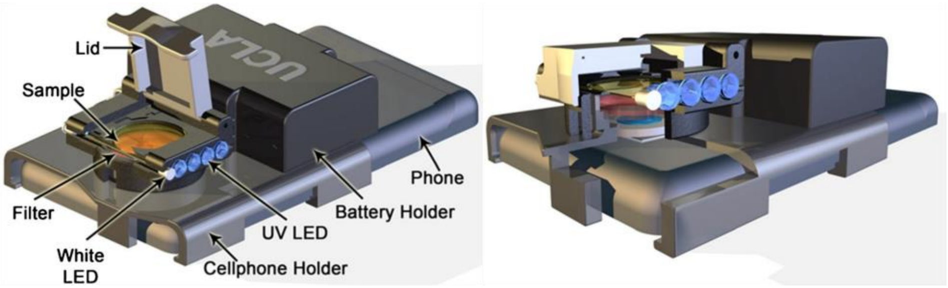

Figure 2. Schematic overview of the cellphone attachment for fluorescence diagnostics developed by Ludwig et al. [14]. Adapted with permission of Springer.

Mora et al. [13] developed a biosensor to accurately quantify lactose or galactose in undiluted food samples using genetically modified bacteria (E. coli) engineered to fluoresce in response to the analyte to reveal its diffusion behavior when using a blue-light source and optical filter. The authors reported detection limit concentrations in the range of 1–1000 mM requiring a sample volume of 1–10 µL and a storability of at least seven days at 4 °C without losing functionality. Bacteria possibily could be reprogrammed to serve as biosensors for other molecules. In this case, the smartphone is proposed as an alternative to visual inspection to quantify the fluorescent read-out.

Smartphone-Based Colorimetric Readers

Colorimetric assay is widely used in biochemistry to test for the presence of several analytes of interest, such as enzymes, antibodies, and peptides. It works by measuring the amount of light absorbed by a chromogenic reagent or a reaction product at a characteristic wavelength. This wavelength is specific to the reagent being measured. The amount of absorbed light is proportional to the concentration of reagent present in the assay well. Different solutions must be made, including a control solution for reference. In this section, the most used architecture is a smartphone camera used to detect the read-out from assay reactions.

Coskun et al. [14] presented a cellphone-based system for colorimetric assays performed in tubes toward sensitive and specific detection of peanut allergen in food samples. They developed a cellphone attachment composed of two tubes, for the test and control solution, illuminated by two LEDs, whose wavelength matches the absorbance wavelength of the reagent activated in the test tube. The light intensity was measured by a dedicated application developed on the smartphone, to quantify the allergen concentration, after a calibration was performed with known concentrations of analyte within the test tube. The colorimetric assays were conducted based on an ELISA test kit specific to peanuts. A 20-min preparation phase was described for sampling and treatment of the target food sample to be ready for the digital reader implemented on the cellphone. The separate optical readout, optimized illumination and imaging configuration resulted to be sensitive, robust, repeatable and immune from manual reading errors compared to visual inspection which can be subject to variable light conditions. Weighing approximately 40 g, this digital tool was able to quantify peanut contamination in food samples with a minimum detection level of ~1 parts per million (ppm) (Figure 3).

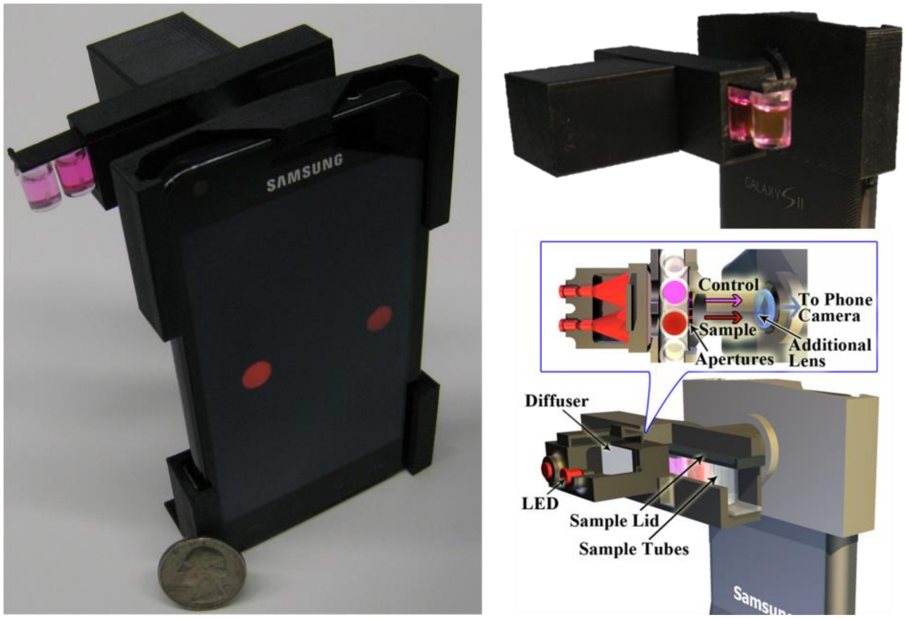

Figure 3. The iTube platform for performing cellphone-based colorimetric assays developed by Coskun et al. Adapted from [16] DOI: 10.1039/c2lc41152k with permission from The Royal Society of Chemistry. All rights reserved.

Lee et al. [15] developed a simple, rapid, and accurate smartphone-based lateral flow immunoassay (LFIA) reader for diagnosis of aflatoxin-B1 in maize. Aflatoxins are toxic secondary metabolites produced by a species of corn fungi. The Lateral Flow Immunoassay (LFIA) is a paper strip-based method for the detection and quantification of analytes. A liquid sample containing the analyte of interest moves under capillary action through various zones of strip, on which molecules that can interact with the analyte are attached. Starting from one end, the sample flows along the strip and it is bound by specific antibodies conjugated with colored or fluorescent particles, finally arriving at the other end of detection in which the recognition takes place, whose read-out is detectable by visual inspection or dedicated reader. In this work, a Samsung Galaxy S2 Smartphone is used as LFIA reader together with a close-up lens and a white LED, to improve the detection limit and sensitivity of the LFIA for AFB1 in maize, minimizing the read-out errors caused by visual inspection. The final result did not rely on the subjective interpretation of an operator. Combining microfluidics and competitive ELISA, Chen et al. [16] developed a smartphone-based portable system for the mobile detection of BDE-47, a common environmental contaminant in food samples. Through the USB port, the smartphone powered an Arduino Nano microcontroller integrated with a PCB, which in turn drove current to a microfluidic-based ELISA chip, triggering the analyte-mediated reaction. The colorimetric read-out was then acquired by the smartphone camera, and the image is then wirelessly transferred to a computing server for post-processing. This lab-on-chip assay showed a significant faster readout time of 15 min compared to conventional ELISAs which typically take at least 2 h. The input sample volume was considerably reduced with respect to laboratory ELISA. This allows the device to be field-deployable in a point-of-care to analyse less-than-ideal samples that the conventional method is insensitive and incapable of detecting (Figure 4).

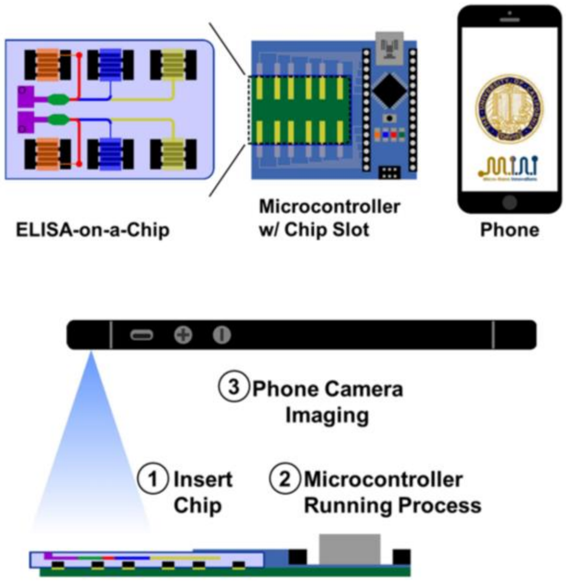

Figure 4. Schematic of the integrated mobile-interfaced diagnostic platform developed by Chen et al. Reprinted from [18], with the permission of AIP Publishing.

Park et al. [17] proposed a portable quality-control method for appraising red wine properties, by means of paper microfluidic channels and colorimetric assay performed with chemical dyes. Six different red wines were used as “model sample set” and four red wines were tested as “evaluation sample set”. A smartphone was used to acquire and analyse the colorimetric result, later processed by means of PCA analysis.

Successful distinction of red wines by their grape varieties and oxidation was accomplished. PC1 was interpreted as explaining the sweetness (sweet or dry), while PC2 the body (light or heavy) of red wine. Minimization of sample-to-sample variation by splitting a single, undiluted red wine sample into eight different wells and filtering particulate matters by paper improved the reproducibility and led to smaller errors, resulting in better separation in the PCA plot. Such image processing and PCA can eventually be implemented as a stand-alone smartphone application, or within a cloud computing environment. Bueno et al. [18] developed a non-destructive method to discriminate three amines, as a diagnostic approach to detect foodborne pathogens in meat, combining membrane technology, dyes, chemometric tools and smartphone technology. A colorimetric test was evaluated using a smartphone and unsupervised chemometric tools, PCA and HCA, achieving a detection limit down to 1 ppm concentration of amine. To demonstrate the effectiveness of the proposed system in a real sample, sample meat were adulterated with amines and then analysed, but to infer the presence of bacteria, authors foresaw the necessity to test the biogenic amine production profile. With the aim of detecting antibiotic residues in milk, Masawat et al. [19] described the development of a smartphone-based digital image colorimeter. Although this work is not properly based on the use of biosensors, the analysis proposed here involved a sample pre-treatment procedure by using invasive Solid-Phase Extraction (SPE) technique to isolate and concentrate analyte of interest, tetracycline (TC), from the liquid matrix in which was dissolved. To protect the system from outside light, a photography lightbox was made with the internal walls sprayed with black paint. The TC solution filled in a quartz cuvette was located in a sample cell holder under fluorescent light inside the lightbox. An iPhone model was used for capturing digital images from outside the box via a drill hole, and the ColorConc application was used to analyze the images. A software calibration phase was performed with a set of images obtained from reference concentrations. Thus, the Euclidean distance algorithm was used to find the closest match to the given image, to identify sample concentration.

Results were compared with double-beam UV–Vis Spectrophotometer. Obtained Limit of Detection (LOD) and Limit of Quantitation (LOQ) for TC concentration measurement, 0.50 and 1.50 μg mL−1, respectively, were higher than the Maximum Residue Limit (MRL) of TC in milk (0.1 μg mL−1). Thus, the pre-concentration of the sample in milk with SPE is necessary to detect TC at low concentration, with the effect of losing portability and ease of use of the proposed system. Monosik et al. [20] presented a paper-based colorimetric assay for the analysis of selected food compounds, instant soups and wines. Food samples were treated with a glutamate-specific enzyme, and colorimetric analyses were conducted after taking a picture with a smartphone, using freeware ImageJ. As a result, a limit of detection of 0.028 mmol L−1 was obtained, while for the naked eye the limit was 0.05 mmol L−1. The versatility of the proposed approach was demonstrated by using other enzymes from the same family. The described method did not require sophisticated approaches in terms of paper pre-treatment and very low volumes of sample and reagents are necessary for the analysis. Yu et al. [21] developed a portable sensing device for pathogen indirect detection based on a disposable lateral flow-through strip sensitive to alkaline phosphatase (ALP). ALP is an enzyme present in raw milk. It is slightly less labile to heat than most pathogenic bacteria; thus, loss of ALP activity is used to confirm proper pasteurization of skimmed or whole milk. The sample solution containing a desired concentration was added onto the sample pad. The read-out on the strip testing zone was imaged by a smartphone-integrated digital camera to quantify the optical signal. The images then were further analysed by a home-programmed MATLAB code. A trace amount of ALP as low as 0.1 U L−1 was distinguished within 10 min, with a detection range of 0.1–150 U L−1. Since the MATLAB code can be programmed to a mobile app, the analysis routine can be automated and performed on smartphone. Okadaic acid (OA) and saxitoxin (STX) are common marine toxins that can accumulate in shellfish and can affect human health through the food chain. To avoid poisoning incidents, Fang et al. [22] proposed an on-site diagnostic platform using a smartphone with competitive immunoassay strips. A smartphone was employed for image acquisition and data processing. A 3D-printed portable accessory of the smartphone was used to fix the test strips. A homemade app was implemented for analysis. First, calibration curves were established with the target analytes diluted into five values of concentration. Then, real sample experiments were performed using the homemade strips for OA and STX and a commercial kit. By means of the strip adapter, the read-out was acquired using an optimized setup for light collection by the smartphone camera and analyzed. The proposed method showed a detection limit of 2.800 ng mL−1 for OA and 9.808 ng mL−1 for STX, similar to those of the commercial plate kit. The entire test time was 30 min, and the system was easy to operate, allowing on-site analysis with a low response time (Figure 5).

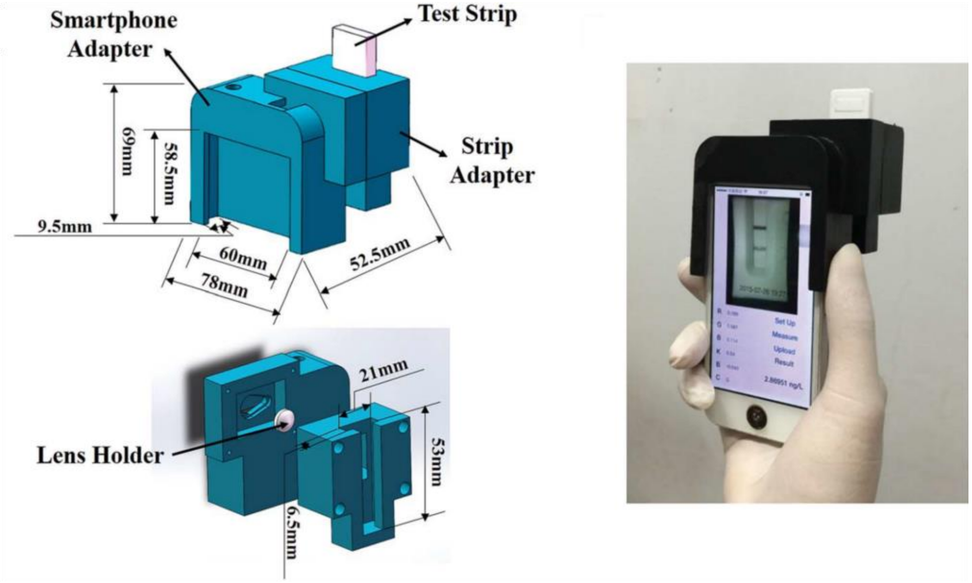

Figure 5. The on-site marine toxins diagnostic adapter developed by Fang et al. Adapted from [24] DOI: 10.1039/c2lc41152k with permission from The Royal Society of Chemistry. All rights reserved.

Driven by the Indian issue of endemic fluorosis, a chronic disease resulting from excess intake of fluoride, Levin et al. [23] presented a field deployable colorimeter for screening of groundwater for fluoride in endemic areas. The proposed method used a commercially available reagent and adapted a smartphone as a colorimeter. An easy-fit, compact, sample chamber adapter for a smartphone was designed to optimize the colorimetric reading. The authors used three smartphones; calibration of each phone was necessary because of significant variation in colour sensing between different camera hardware. The calibration process involved analysing five different fluoride standards. A software program was developed to use with the phone for recording and analysing the RGB colour of the picture. The resulting images were analysed using a linear interpolation to calculate the expected colours in between the calibrated colours. The linear range for fluoride estimation was 0–2 mg L−1; results were comparable with those of expensive laboratory Ion Selective Electrode reference method, without the need for technical expertise to conduct the test analysis (Figure 6).

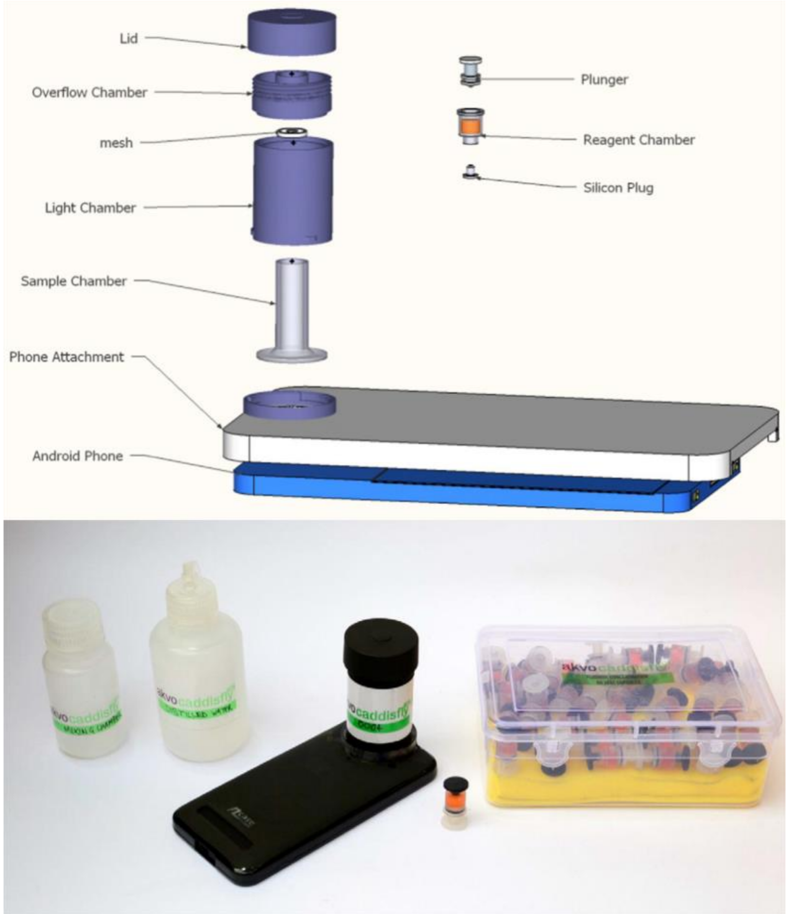

Figure 6. Schematic and picture of the smartphone-based fluoride test proposed by Levin et al. Adapted from [25], http://dx.doi.org/10.1016/j.scitotenv.2016.01.156 under the Creative Commons license http://creativecommons.org/licenses/by/4.0/.

Wang et al. [24] developed a smartphone-based colorimetric reader coupled with a remote server for rapid on-site analysis of catechols, an environmental pollutant highly toxic by ingestion and contact, which can irritate the human eyes and skin, and even at low concentrations, can give foods an undesirable taste. A 96-well sensor array was inserted in a light-tight box between a white LED light in the bottom and a smartphone fixed to the top. With this equipment, the ambient lighting condition and imaging distance/angle were kept constant when capturing the images of the sensor array. Thirteen different catechols at six serial concentrations were evaluated for system calibration using PCA, HCA and LDA for quality discrimination and PLS for quantitative determination. Data were uploaded to a remote server to form analysis polynomials for LDA and PLS of an unknown sample. Real water sample analysis was performed, with very good estimation results achieved. The authors claim this work to be the first dealing with the on-site detection of analytes using a smartphone-based colorimetric reader coupled to a remote server. With the aim of separating the detection means from the phone to resolve the difficulties in applying different models of mobile devices to the field test, Seo et al. [25] realized a pocket-sized immunosensor system for the on-site detection of foodborne pathogenic bacteria. The immunoassay procedure was based on chemiluminometric signal generation. The biosensor cartridge included a lens-free CMOS image sensor (CIS) physically contacting the signal generation part of the cartridge and Wi-Fi module installed in the circuit board. The system was controlled by a smartphone app programmed by the authors. The internet-of-things (IoT) technique was intended for use in food contamination monitoring and was demonstrated by analyzing V. parahaemolyticus present on fish samples and uploading the data to a server via a wireless network. Prior to food testing, the target bacterium was pre-cultivated. The cultured medium then was analysed by employing the immunosensor system controlled by the mobile device, and the result was uploaded as information to an internet server. A LoD of 1.4 × 104 CFU mL−1 was achieved. Such a technique combining a biosensor with IoT can be used to issue a warning immediately after complete analysis about food contamination before purchase or consumption, so that the supply chain can be promptly blocked. The authors claim the study to be the first exemplification of pathogen monitoring via IoT. Finally, DuVall et al. [26] presented a rapid detection of foodborne pathogens using a cell phone and custom-written app, in which the physical identification was made by pathogen DNA transduction, mediated by magnetic bead aggregation with pathogenic DNA fragments. The smartphone was used to acquire picture of the assay reaction and analyse the image to perform a qualitative Yes or No detection of pathogen presence. The proposed detection modality was fully portable for point-of-care detection of food-borne pathogens Escherichia coli O157:H7 and Salmonella enterica.

Smartphone-Based Electro-Analytical Platforms

Electroanalytical methods use electrodes to make electrical contact with the analyte solution, in conjunction with electric or electronic devices to which they are attached, to measure an electrical parameter of the solution. The measured parameter is related to the quantity of an analyte in solution. According to the electric parameters that are measured, electroanalytical methods include potentiometry, amperometry, conductometry, electrogravimetry, voltammetry and coulometry. The names of the methods reflect the measured electric property or its units. Electroanalytical methods are particularly interesting for the development of smartphone-based platforms for on-site food diagnostics, as they combine high-performance detection with great simplicity, low-cost, portability, autonomy, cable-free operation, and capacity to conduct in real-time the entire analytical measurement at remote places.

Dou et al. [27] described a biosensing system for the detection of clenbuterol (CLB), using a mobile electrochemical device with an electric field-driven acceleration strategy. CLB has been illegally used in livestock raising to improve growth rate, reduce fat deposition and increase protein accretion. However it has been banned as a feed additive in foodproducing animals in most countries because it can easily remain in animal tissues and result in clinical symptom in human such as temporary dizziness and palpitations. The electric field-driven method was selected to accelerate the immunoreaction at the solid-liquid interface of electrodes, speeding up the transport of low-abundant drug molecules. A smartphone tool biochip was developed to conduct the electrochemical detection and send data to the phone via USB port. The smartphone-based immunosensor was able to detect a minimum of 0.076 ng mL−1 CLB in 6 min. The advantage of this method is that, by combining different functionalized electrodes, this device can meet the requirements for field detection of all food security-related species. In another study, Giordano et al. [28] coupled a homemade potentiostat to a mobile phone for point-of-use assays successfully applied for pattern recognition of Brazilian honey samples according to their botanical and geographic origins. The method relied on the unsupervised technique of principal component analysis (PCA), and the assays were performed by cyclic voltammetry using a working electrode of gold. The proposed biosensor platform was provided with both USB connection and Bluetooth module integrated in the potentiostat hardware. An in-house app was developed to ensure the on-site processing of multivariate data using PCA. The system was also created with the possibility to share data through the cloud (e-mail, Google drive, or even social media) for backup or remote processing of the electroanalytical results with more advanced chemometric tools. The authors claim that this is the first reported work concerning the development of a totally integrated point-of-use system with chemometric data processing on a smartphone (Figure 7).

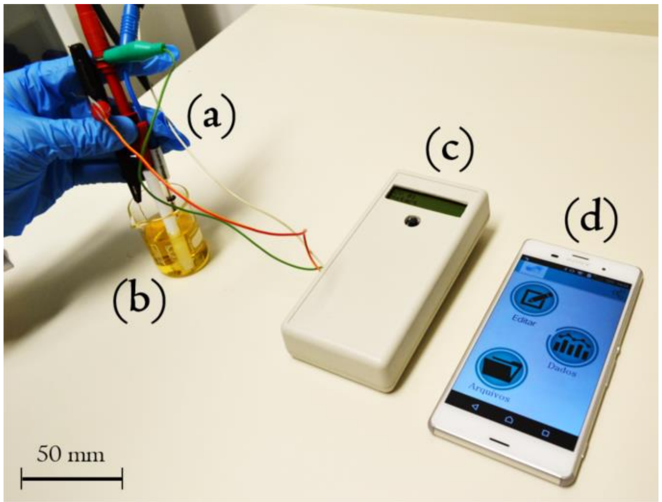

Figure 7. Portable platform deployed for point-of-use analyses. Electrochemical system. (**a**) Sample; (**b**) hand-held potentiostat; (**c**) and smartphone; (**d**) Reprinted from [30] with permission from Elsevier. http://www.sciencedirect.com/science/article/pii/S0013468616320400.

2) *Smartphone Spectroscopy*

The works reviewed in this section belong to the optical diagnostics macro-category. Unlike the approach based on biosensors, in which it is necessary a reagent to trigger the transduction, in this case the analysis is performed in a non-invasive manner. In particular, spectroscopy has been a powerful tool in research and industrial applications. It is extensively and successfully used in applications including diagnostics, assessment of food quality, environmental sensing, and drug analysis testing. This technique is intrinsically rapid and non-destructive. However, most spectrometer setups used in industrial or laboratory-based applications are expensive and bulky, limiting them to controlled laboratory settings. Recently, due to advancements in electronics and fabrication methods, more portable spectrometers have been realized. Technological progress has allowed the release of micro-spectrometers which take advantage of new micro-technologies such as microelectromechanical systems (MEMS), micro-opto-electromechanical systems (MOEMS), micro-mirror arrays, etc. These improvements reduce cost and size while allowing good performance and high-volume manufacturability. Compared to lab-based instruments, miniaturized systems must become a black-box, providing expected results with high reliability and without intervention of technicians specialized in spectroscopy measurements. The ultimate goal, in the future, is the integration of a spectrometer into a smartphone, taking advantage of the highly efficient processing abilities in the compact configuration, to offer spectroscopic information on the fly. Moreover, they will use the large community of users to build databanks based on machine learning through apps [29]. A detailed overview of the revised Smartphone Spectroscopy works is provided with Table 2.

Table 2. Summary of recent smartphone spectroscopy systems.

Detection Target	**Methodology**	**Materials**	**LoD/Performance**	**Smartphone Use**	**Ref.**
Microbial spoilage on beef	Mie scattering	Positioning stages, 880 nm NIR LED	10^1 CFU mL^{-1} to 10^8 CFU mL^{-1}	Built-in gyro sensor and camera spectroscopy	[33]
Generic application	Spectroscopic colorimetry	3D printed housing, LED array, Phidgets board, and VIS-spectrometer	Good agreement to certified spectra with dE/E ranging from 0.5% to 1.5%	IoT device to be used with smartphone	[34]
Glucose and ethanol in alcoholic beverages	FTIR spectroscopy and independent component analysis	Graphite light source, ATR prisms, 2-dimensional light receiving device for smartphone	Wavelength resolution 0.057 μm	Proposed as a bean-size spectroscopic module to be mounted on smartphones	[35]
ChlF detection in a variety of apple samples	UV fluorescence spectroscopy	UV LED, nozzle-like enclosure VIS-spectrometer, Arduino pro mini μ, Bluetooth	Satisfactory agreement observed between ripeness and fluorescence signals	Dedicated app interface on smartphone to communicate, receive, plot, and analyse spectral data	[36]
E. coli contamination on meat	Fluorescence-based imaging	4405-nm 10 W LEDs, CCD camera, optical filter at 670 nm, and Wi-Fi transmitter	Localization of most fecal contamination spots successfully identified	Outlined real-time broadcasting to monitoring device such as smartphone	[37]

Generic food sensing application	Hyper-spectral imaging	Tunable MEMS FPI, Bluetooth	Operation range 450–550 nm with spectral resolution 8–15 nm @FWHM	Mobile phone-compatible hyper-spectral imager	[38]
Food quality testing	Diffractive interference refractometry	5 mW semiconductor red laser, circular spatial filter, Si detectors, and a PDMS device	LoD of 4×10^{-4} RIU	Outlined smartphone interface based on transmission mode configuration	[39]
Sugar content prediction in pears	NIR spectrometry and PLS	4 tungsten lamps, LVF 620–1080 nm and CMOS linear detector array	Low power, SNR ratio up to 5000, R^2 0.96, SEC 0.29° Bx and SEP 0.46° Bx	Instrument wirelessly operated with smartphone	[40]

Liang et al. [30] proposed a detection method for microbial spoilage of beef by means of a smartphone-based optical diagnostic system. An 880 nm near infrared (NIR) LED was irradiated perpendicular to the surface of ground beef, while the digital camera of a smartphone detected the scatter signal angled at 15°, 30°, 40°, and 60° from the incident light. Experiments were performed with and without positioning stages, where in the latter case, a software application and the built-in gyro sensor of the smartphone were used to control the incidence angle between the iPhone camera and the NIR LED light source. Concentrations of E. coli (from 101 CFU/mL to 108 CFU/mL) were determined by the "pattern" of such scatter intensities over the angles. The proposed device was presented as a preliminary screening tool to monitor microbial contamination of meat products. Mignani et al. [31] presented the proof-of-concept of SpiderSpec, a compact colorimeter composed of a 3D printed cylindrical housing containing a LED array for illumination and a compact spectrometer for detection, with food control proposed as a possible application. The 12 visible LEDs were arranged in a circular array in the optical head, with 45° orientation with respect to the central detection axis, which is one of the standard configuration for reflectance measurements. The chosen spectrometer had an operative range of 350–800 nm. A custom Labview software interface was used for managing the LEDs and spectrometer. However, the authors, in a view of configuring the spectroscopic colorimeter as an IoT device, depicted future development of functionalities which could be selected using a smartphone or a tablet (Figure 8).

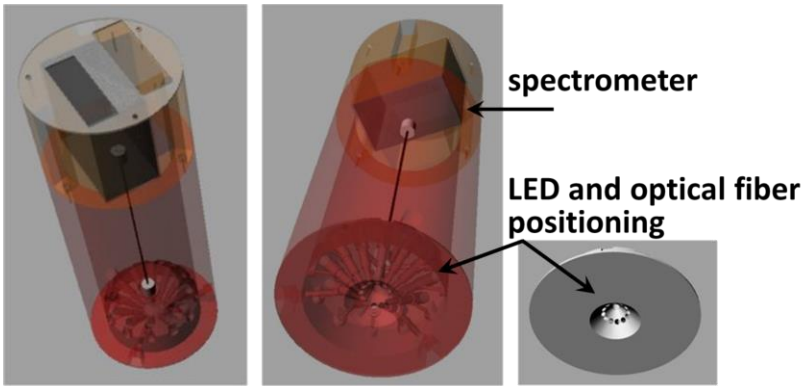

Figure 8. Rendering of the spectrometer-based colorimeter SpiderSpec. Reproducted from [31] with permission from SPIE.

Since conventional Fourier transform infrared (FTIR) spectrometers equipped with Attenuated Total Reflection (ATR) are bulky and expensive apparatus, on-site measurements of foods or drinks on the manufacturing site are impractical. In this perspective, Hosono et al. [32] developed an ultra-compact alkaline battery-size FTIR spectroscopic imager for simultaneous measurement of glucose and ethanol in alcoholic beverages by means of independent component analysis, employing a bean-size spectroscopic module to be mounted on smartphones. Experiments were performed in the NIR and MIR regions to find a range suitable for independent component analysis for discrimination of glucose and ethanol. The first use of a compact standalone spectrometer in combination with a smartphone via wireless connection was by Das et al. [33], which demonstrated the development of a mobile device for fruit ripeness evaluation. The authors used the portable spectrometer prototype to study UV fluorescence of chlorophyll (ChlF) in fruits. ChlF is a good indicator of photosynthetic activity and has been observed to relate to defects, damage, senescence and ripening of post-harvest fruits. Most important, this method enables the detection of fruit ripeness in a non-destructive manner. In this work, the smartphone spectrometer assembly was used to rapidly evaluate ripeness of different varieties of apples using ChlF emission when excited using UV light. UV LED with a wavelength of 360–380 nm was used as excitation source coupled with a spectrometer of range 340–780 nm. A calibration equation was applied to convert pixels to wavelength. Subsequently, a Bluetooth interface was setup to communicate with the smartphone. A customized app was developed for the Android operating system to communicate with the spectrometer assembly, and plot and analyse the spectra on the smartphone. ChlF detection in a variety of apple samples was performed and compared with the reference ripeness estimation using destructive mechanical firmness testing. The proposed device overcame the problem of stray light interference by launching and collecting light through a nozzle-like enclosure, thereby shielding any stray light contribution, a feature essential for field-based applications. However, a limiting factor in the proposed setup was the relatively low ADC bit resolution, property-dependent on the microcontroller choice (Figure 9).

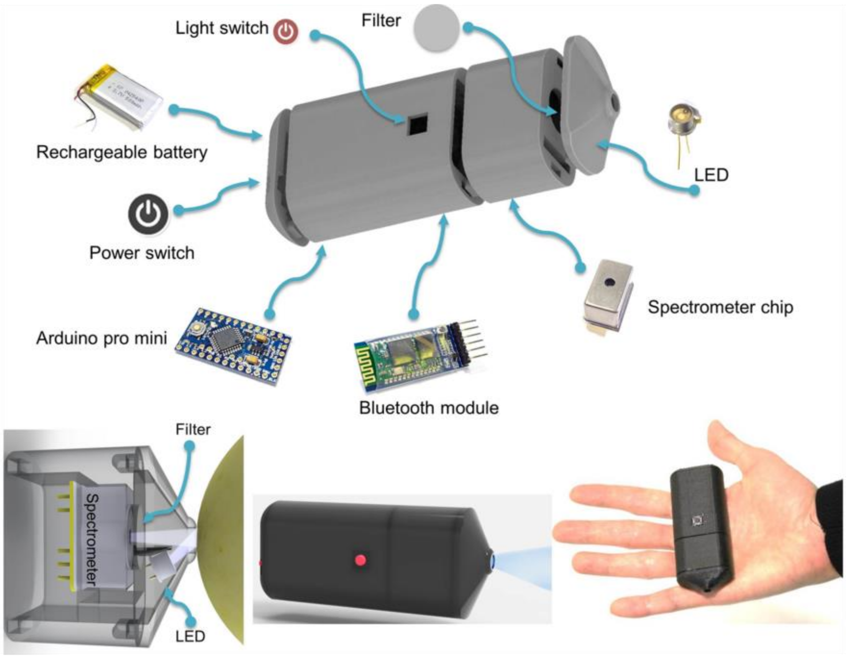

Figure 9. Schematic of the different components of the smartphone spectrometer prototype. Adapted from [36]. Published online 8 September 2016. doi:10.1038/srep32504, under the Creative Commons license http://creativecommons.org/licenses/by/4.0/.

Contaminated foods originating from animal products are a significant source of human infection and illness. Because animal feces are the most likely source of pathogenic E. coli contamination associated with foodborne illnesses, it is particularly important to inspect for fecal contamination on meat during meat processing. Currently, meat inspection in slaughter plants for food safety and quality attributes, including potential fecal contamination, is conducted by visual examination from human inspectors. Oh et al. developed a handheld fluorescence-based imaging device to be an assistive tool for human inspectors with the aim of enhancing visual detection of fecal contamination on red meat, fat, and bone surfaces of beef under varying luminous intensities [34]. The device comprised four 405-nm 10 W LEDs for fluorescence excitation, a charge-coupled device (CCD) camera, an optical filter at 670 nm, and a Wi-Fi transmitter for sending real-time data to smartphone or tablet. The localization of most fecal contamination spots on beef surfaces was successfully identified because of the presence of chlorophyll metabolites discharging fluorescence near 670 nm. The image acquired from the device was transmitted by Wi-Fi and processed by MATLAB analysis. As expected, the increase in luminous intensities led to a parallel decrease in the identification of the fluorescence spots. Results indicated the proposed system as an effective way to aid visual inspection for fecal contamination detection. Rissanen et al. demonstrated a mobile phone-compatible hyper-spectral imager based on a tunable MEMS Fabry-Perot interferometer for authentication, counterfeit detection, and potential health/wellness and food sensing applications [35]. The authors described the development of a MEMS Fabry-Perot interferometer (FPI) tunable optical filters integrated with an iPhone 5s camera to perform hyper-spectral imaging in the vis-NIR range 450–550 nm. The communication between the MEMS FPI module and iPhone 5 was arranged using Bluetooth. A configuration of two cascaded FPIs (λ = 500 nm and λ = 650 nm) combined with an RGB colour camera showed potential to expand the wavelength tuning range to 400–700 nm. Sasikumar et al. [36] developed a handheld optical analyser consisting of a collimated 5 mW semiconductor red laser (635 nm) as the source, a circular spatial filter, Si detectors, and a Polydimethylsiloxane (PDMS) device. Fabrication of the PDMS device with integrated sample well was adapted for refractometric, and hence concentration, measurements. Potential integration of this device with smartphones was outlined, and featured a simple interface based on transmission mode configuration to explore several applications in food quality testing. Yu et al. realized a handheld NIR spectrometer specifically designed to assess the internal quality of fruit. In particular, a key development aspect was the Linear Variable Filter module as a light-dispersion component [37]. The proposed spectrometer system was a gun-shaped device, operating in the vis-NIR range (620–1080 nm) in interactance mode. The light source consisted of four tungsten lamps placed symmetrically around the entrance window. A 6-mm-diameter rubber grommet surrounded the entrance window and acted as a light seal, thus preventing surface-scattered light from reaching the window directly. A soft black foam ring around the detector head was provided to support the fruit during analysis and shield it from external light. The platform was tested for determining the sugar content in Crown Pear. Light from the source entered the fruit and penetrated part of the tissue, and that which emerged from the fruit entered the window. Results were compared with the reference method of Brix measurements, recorded with a handheld refractometer. Models were developed using PLS regression with the full band of the absorbance spectra and were optimised by applying MSC, SNV, and first derivative. The instrument was able to analyse spectral data using an on-board prediction model and to operate wirelessly with a smartphone, tablet or laptop computer. It proved highly suitable for predicting fruit internal quality. However, modified software is needed, and further studies are required to test the performance of the spectrometer for predicting other attributes or detecting sugar in other fruits (Figure 10).

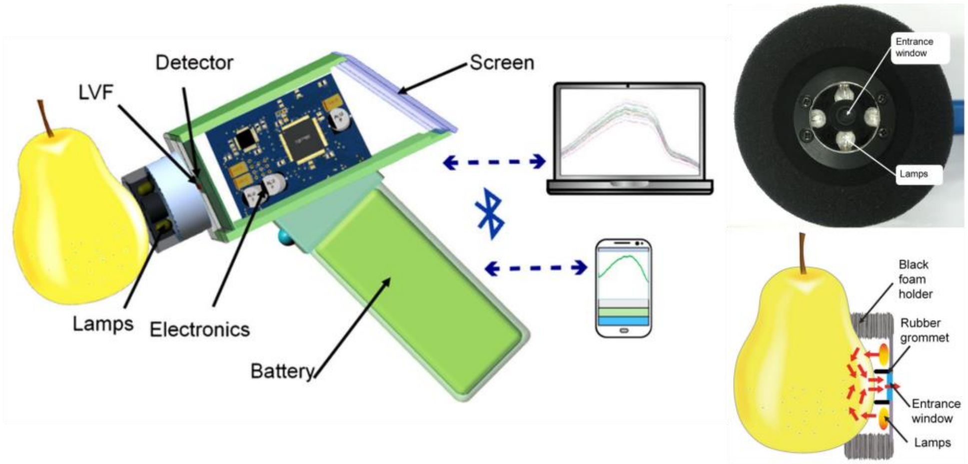

Figure 10. Schematic of the hand-held spectrometer and picture and schematics of the measuring head for interactance mode measurement developed by Yu et al. [40]. Adapted by permission of SAGE Publications, Ltd.

To get an idea of the cost-reduction when switching to smartphone-based sensors, approximate costs are provide with Table 3, for some of the technologies above mentioned.

Table 3. Approximate cost related to works which have provided an estimate.

Platform	Approximate Cost	Ref.
Smartphone with fluorescence microscope attachment	Attachment of around $140, significantly reduced compared to the equipment costs for the reference method	[12]
Akvo Caddisfly	Expected to retail at $75, without the phone and mapping system, plus $0.3 for each test	[23]
PiBA assay coupled to LAMP	Reagent cost for PiBA is a fraction of a cent. Overall cost reduction is ~10-fold respect to the reference (fluorescence reagents for qPCR)	[26]
Smartphone-based analytical platform with homemade potentiostat	Based on CheapStat potentiostat which requires less than eighty dollars for its manufacturing, while the most commercial potentiostats cost a few thousands of dollars	[28]
Smartphone spectrometer	Entire assembly along with the smartphone can be realized under $250, while reference spectrometer platforms costs are $4000 and $1200	[33]

3) *Emerging Market of Smartphone-Based Food Diagnostic Platforms*

Many start-ups that are proposing the use of mobile devices able to test the quality of food and to determine its constituents are emerging, also thanks to the increasing use of the crowdfunding platforms Indiegogo and Kickstarter. These smart systems represent a mobile and miniaturized labs, optimized for the detection of a specific target, which are offered in combination with dedicated smartphone applications that provide friendly user interfaces for handling and displaying the test results, received through BLE (Bluetooth low energy) connection. Moreover, thanks to the ubiquitous smartphone connectivity, they become IoT modules able to leverage the extensive computational power and storage offered by cloud computing. Here, we provide the most relevant products, narrowing the list to those covered by a filed patent.

Cellmic LLC (formerly Holomic LLC), founded by Professor Aydogan Ozcan, offers a suite of rapid diagnostic test readers for advanced mobile diagnostics [38]. Among these is the allergen testing platform already described in this paper [16] and covered by a patent [39]. This device was developed by UCLA researchers for the detection of allergens, based on ELISA kit and a test tubes-containing module attachment to the smartphone camera. MyDx Inc. is a science and technology company that has created MyDx, a handheld electronic analyser that leverages electronic nose nanotechnology to accurately measure chemicals of interest in food and water, to detect traces of pesticides or metals, and send results to smartphone handled by the MyDx app [40]. The company owns many related patents including [41].

Scientists and researchers of Kaunas University of Technology, in cooperation with the company ARS Lab, have developed the patented [42] smart electronic nose FOODsniffer (formerly PERES). It is based on gas sensors and is intended to signal the deterioration of meat and fish by detecting gases that reflect such deterioration. It was selling on the company website for $129.99 at the time of writing [43]. Nimasensor [44], was developed by Nima Labs Inc. and enables the detection of the presence of gluten in food. It is based on the immunosensor technique, in which a specific antibody binds gluten, sparking the transduction process. Therefore, it is necessary to sample the food inside a disposable cartridge that is then inserted into the main body of the device. Results are provided through a dedicated app. The Nima device embeds an OLED display indicating a smile for gluten under 20 ppm. The Nima Starter Kit cost at the time of writing was $279.00 [45]

Many new companies are offering sensors based on the emergent and promising technique of NIR spectroscopy. Spectral Engine Oy presented a plethora of high-tech products based on a tunable optical filter as a peculiar component, originated from years of research done at VTT Technical Research Centre of Finland, which resulted in many filed patents, including [46]. The Wireless NIR sensor device platform, which is designed for portable applications development, can be operated with a computer, a tablet, and a smartphone. The Food Scanner solution concept uses the wireless NIR sensor in conjunction with advanced algorithms, cloud-connectivity, and a vast material library to reveal the fat, protein, sugar, and total energy content of food items with a good level of accuracy [47]. TellSpec Inc. proposed a pocket-sized NIR spectrometer, a cloud-based patented [48] analysis engine, and a mobile app that work together to scan foods; identify calories, macronutrients, allergens, and contaminants; and provide relevant information such as food fraud, food adulteration, and food quality. The spectrometer is based on the Texas Instruments DLP® NIRscan™ technology. At the time of writing, two solutions are currently delivered on the company website, the Enterprise Scanner at $1300.00 and the Software Development Kit at $2000.00 [49]. Finally, with several filed patents including the [50], the Israeli company Consumer Physics (formerly Verifood Ltd.), has the in-house-developed product, SCIO, a pocket-sized NIR spectrometer for molecular analysis, including food. It is delivered in the solutions of Consumer Edition at $299.00 and SDK at $499.00 at the time of writing. Moreover, in partnership with Changhong and Analog Devices, the company has just announced the world’s first Molecular Sensing Smartphone, a smartphone integrating the Scio spectrometer module [51]. The presented commercial devices are depicted in order of the description above in Figure 11.

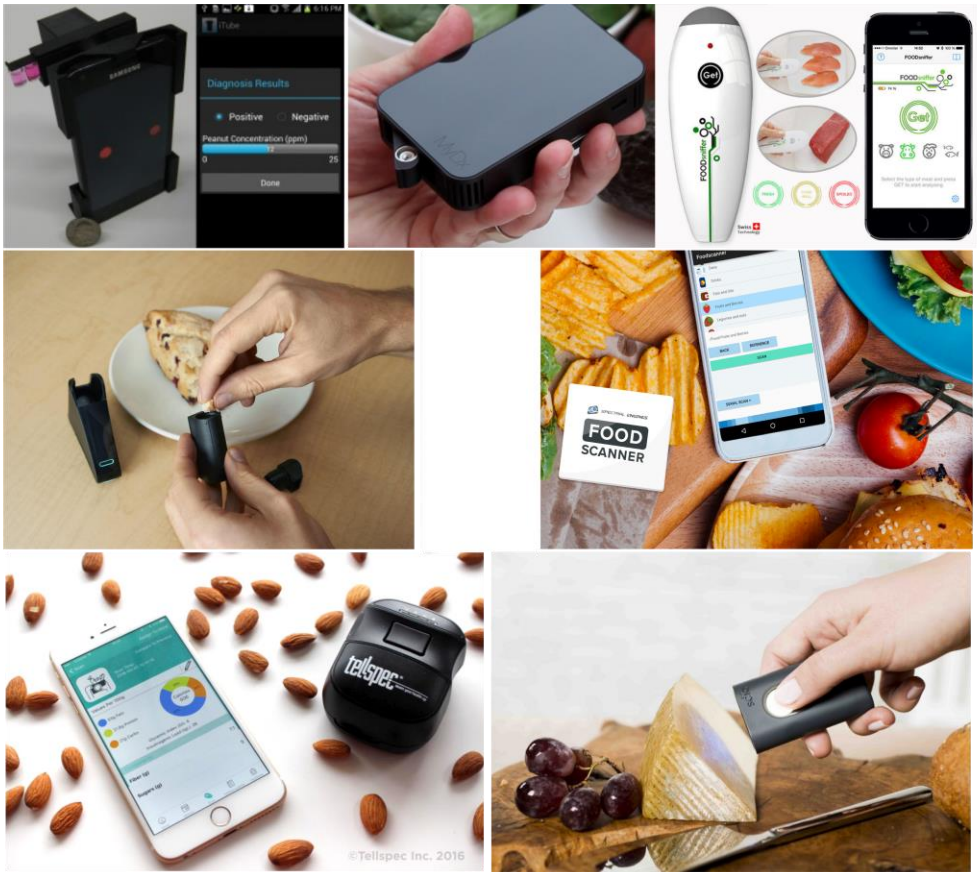

Figure 11. Commercial products for mobile food diagnostics.

Conclusions

The proposed sensing strategies, which used a phone as the read-out tool (e.g., colorimetric and fluorescence imaging), are optimized for the phone models used for carried out the analysis. In order to meet the property of repeatability between different platforms, calibration of each phone is necessary because there is significant variation in color profile between different phones, due to hardware differences. Indeed, cameras may have different spectral responsivities, lamps may have different spectral emittances, and digitizer elements may be different. Moreover, the above mentioned properties may change over time. Another point to be addressed when dealing with pictures and colors is the image format. As smartphone cameras have become more and more powerful, it is now possible to shot in lossless RAW format, instead of lossy JPEG. In scientific imaging, there are several reasons to choose the first one. When shooting in JPEG image information is compressed and lost. The camera does its own processing to convert into a JPEG. The white balance and colour space are applied to the image by default. With RAW, within the image all data from sensor are recorded, so it is always possible to perform post-processing, like adjusting white balance and selecting the proper colour space where to export out the picture.

Sample preparation is still a bottleneck for the field of food mobile diagnostics, which aim is to bypass the use of expensive and bulky instrumentation-based tests, operated by trained personnel. Sampling performed by non-expert user may lead to unwanted contamination, resulting in defiled measurements. For example, in colorimetric assay, if unwanted solutes in the sample buffers positively or negatively affect light absorbance, it results respectively in false positives or negatives. This intrinsic drawback affects mostly detection strategies in which sampling is a necessary preliminary step. The commercial systems presented have tried to overcome this problem through a user-friendly design, inserting guidelines to assist customer during sampling and calibration procedure.

The methodologies discussed indirectly identify the concentration of a target substance, so for all of them it is necessary to make a calibration with a reference standard, using the pure substance at different concentrations, in order to build the instrument calibration curve for that specifically target. All conditions under which standards and unknowns are prepared should be kept identical. For fluorescence, dye- and glass-based reference materials are used to correct fluorescence emission for relative intensity, comparing an unknown measured intensity value with the certified values. In colorimetric assay, to correctly identify unknown samples, first the software module should be calibrated with a set of images obtained from reference concentrations. Each image will be associated with a concentration level. In case of electro-analytical techniques, the measured electrical quantity is proportional to the concentration of some component of the analyte. Calibration is done using standard buffer solutions at different known concentrations, developing the transduction characteristics. Reflectance spectroscopy needs calibration against a reference method with the ingredient of interest, to associate to a spectra a quantitative information. In the case of non-invasive analysis, such as reflectance spectroscopy, sampling is less important than performing a correct workflow for measurements. In the future, it is hoped that these systems will be able to self-calibrate. Non-invasive and machine learning-based systems, which rather than following standard calibration using reference concentrations, can count on huge amount of data, are definitely favourites from this point of view. Indeed, smartphone-based diagnostics allows ease capture of data and generation of large datasets, which can be appropriately managed by means of advanced computational analytics, such as machine/deep learning in combination with human expertise, for the extraction of meaningful information [52].

All above described studies and commercial products show that this novel field of research represents a promising area that has high scientific and commercial impact. In particular, advancements in biomedical science, chemistry, biotechnology, optics, and engineering have led to new diagnostic platforms which are more portable, economical and easier to use than conventional lab-based assays. Furthermore, the universal presence of mobile phones in our society makes it possible to leverage these devices for on-site testing. Nevertheless, these systems raise questions about use protocols and reliability of measurements. The repeatability of a measure, intrinsically guaranteed by a laboratory apparatus, becomes a delicate condition to be met in case of portable modules for on-site analysis. Opportune optimizations must be evaluated at the design stage for the physicality of the instrument, to exclude or minimize any external noise sources.

Researchers have come up with different solutions and embodiments for exploiting the great potential offered by smartphones. According to the detection strategy, we have classified the revised works into two main classes; biosensor-, and spectroscopy-based smartphone platforms. In both of these approaches, mobile phone provided a simplified user interface, visual display, data processing, storage, and wireless transmission. While both strategies feature advantages, they also face several limitations. Biosensors are economic and intrinsically sensitive to a specific target and only need slight computation processes. Nevertheless, they are based on disposable cartridges or strips, and require invasive sampling to perform the diagnostics. Spectroscopy indeed allows peculiar rapid non-invasive and non-destructive analysis. However, it is not a target-specific method and it must be associated with complex multivariate statistical and chemometric tools for spectral dataset analysis to extract the relevant chemical information. While in the biosensors based-approach, the key-enabling factors are the choices of the reagent and the transduction process, in case of spectroscopy, the critical design criteria are represented by the selections of the wavelength range for source and detector, and of the measurement setup. Thus, both approaches are easily adaptable methods, since calibration and tuning of the systems are performed according to the desired application. Moreover, they require only a basic training, and a few minutes for detecting and processing, with the potential for providing a user-friendly, on-the-go, scanning scenario. While lab-on-smartphone biosensor applications are well established, the exploitation of smartphone spectroscopy is in its infancy. Fortuitously, advancements in the fabrication of optical sensors, which are leading to increasingly miniaturized and economical technology, are keeping pace with the development of increasingly sophisticated machine-learning algorithms. This, combined with the enormous potential offered by cloud computing, and the ability of modern smartphones to act as both connecting portals and interfaces for analysis and display of results, dramatic developments are foreseen in the field of mobile diagnostics, operated not only for food monitoring applications, but also for environmental and biomedical sensing.

B. Objective of this Thesis

Aim of this work will be the detailed study of the Near-Infrared Spectroscopy methodology and its exploitation for the development of non-invasive optical analysis solutions for nutrition monitoring, food scanning and industrial applications. After an in-depth analysis of the state of the art of portable food analysis enabling technologies previously described in the Introduction, this methodology has been selected for its countless advantages, which will be described below, which make it a very promising solution in terms of range of applicability.

In Chapter II a multi-platform optimized analytical method based on the most informative wavelength range in the NIR region for quantitative measurement of sugar content in aqueous solutions and beverages by means of absorbance spectroscopy will be described. In Chapter III, exploiting the preparatory results achieved in the previous Chapter, the design, development and testing of a LED-based optical and portable platform for sugar content measurement in beverages will be shown. Finally, in a view to highlight the wide range of applicability of portable spectroscopy, in Chapter IV will be illustrated how near infrared reflectance spectroscopy has been proposed and implemented to empower a robotic arm with material classification capabilities, for in-line automated sorting of waste.

II. MULTI-PLATFORM SUGAR CONTENT MEASUREMENT IN AQUEOUS SOLUTIONS AND BEVERAGES

In this Chapter a multi-platform optimized analytical method for quantitative measurement of sugar content in aqueous solutions and beverages via absorbance spectroscopy is described, based on the most informative wavelength range in the NIR region.

A. *Introduction*

Spectroscopy is a fast, non-invasive, and non-destructive analytical methodology, widely used in diagnostics, in the assessment of food quality, environmental sensing, drug analysis and testing [53]. Near-infrared spectroscopy has the advantages to be a non-destructive and greener alternative to reagent-based techniques analytical technique; no need for reagents and limited waste after the analysis. Moreover, there is a reduced or null sample preparation, and it allows the possibility of multicomponent analysis in real time. The payoff in using this technique is enormous in terms of allowing rapid, non-invasive, low-cost analysis on-site, thus eliminating the need of sending samples for time- and budget-consuming lab testing. Although the effort to obtain a reliable prediction model might seem a huge up-front investment in time. Indeed, unlike infrared absorbance spectra, in which the Beer-Lambert law can be used to determine an unknown concentration evaluating absorbance at a specific wavelength, NIR absorbance spectra cannot be directly analysed, but need to be pre-processed using chemometric statistical analysis according to the specific application, to extract meaningful chemical information. Chemometrics is the interface between analytical chemistry and mathematics. It is the application of mathematical and statistical method to extract chemical information from measured spectroscopic data [54]. For classification purpose, it could be used to perform pattern recognition inside big dataset deriving from NIR spectroscopic analysis of multiple materials, exploiting NIR spectroscopy peculiar ability to generate material ‘fingerprint’ [55]. For quantification purpose, as happens by the application of Beer-Lambert law, chemometrics prediction model requires large dataset of spectra acquired from samples containing different concentrations of an analyte of interest. Many studies have directed effort towards the implementation of method able to identify variables which carry useful information and in parallel eliminate variables containing mostly noise [56]. In [57] a variable selection methodology was proposed to determine the most informative wavelengths to build a Multiple Linear Regression (MLR) model for water status of plants. In [58] a wavelength selection method was described, to identify the three most informative wavelengths for grape ripeness estimation. A quantitative model for three sugars in bayberry juice was developed using PLS regression and was provided in [59]. Indeed, NIR spectra are sensitive to the presence of organic compounds which contain the molecular bonds C–H, O–H, and N–H. In particular, sugar molecules contain C–H, O–H, C–C, and C–O bonds and related absorption mainly came from the third overtone and second overtone of C–H and O–H bonds. An absorption peak around 970 nm is due to the existence of the O–H stretching vibration and water [60].

B. State of the art

As prior art for this work, articles regarding proposal of wavelength selection methods as advanced chemometrics approach to build prediction quantitative model for sugar content in liquid food have been considered. Moreover, the focus has been further narrowed down to the "silicon range" of Visible-NIR detectors (up to 1000 nm), which technologic development combined with development of more and more sophisticated machine learning algorithms can lead to increasing miniaturized, cheap and reliable platforms, with possible integration with smartphones [61]. Following the rapid development of chemometrics research, NIR spectroscopy has been more and more applied to the analysis of liquid foods [62]. The major benefit of working with liquid samples is the handling of optical path length. In case of a liquid with low scattering characteristics, the use of a cuvette to contain the samples provides high consistency in optical path length, with measurements performed in transmittance mode. [63]. On the basis of Partial Least Square (PLS) regression coefficient, authors of [64] have analysed the fingerprint reflecting characteristics of visNIR orange juice spectra for soluble solid content (SSC) -solids concentration of a sucrose containing solution-, highlighting which wavelengths might be of particular importance for calibration. In [65] sensitive wavelengths corresponding to the SSC of bayberry juices were proposed on the basis of regression coefficients by PLS, implemented on Vis/NIR spectra. In [66] PLS have been used to build regression model for soluble solid content measurement in tea soft drinks and also to extract the most sensitive wavelength, which were then exploited to build a MLR model. In [67] researchers have extracted sensitive wavelengths for reducing sugar determination in fermenting grape, analysing loading weights of latent variables from the PLS model, used then to build a MLR model. Researchers in [68] have studied three variable selection criteria for determining SSC in beer. In [69] a variable selection based on interval-PLS (iPLS) algorithm was implemented to determine Sugar content in commercial plant milks. To the authors knowledge, there is a lack in literature regarding the realization of a reliable quantitative method based on variable selection for sugar concentration measurement in the NIR range exploitable on many systems. To fill this gap, in this work we propose a multi-platform optimized approach of developing a sugar concentration prediction model in liquids. Spectral dataset from aqueous solutions of three sugars were acquired using a benchtop instrument and a mini-spectrometer in the range 600-1100 nm. With proper pre-processing of raw absorbance spectra, the most sensitive wavelengths for sugar content were highlighted for each setup. Thereafter, a regression algorithm has been calculated and validation has been then performed with commercial soft drinks containing different known sugar content. A discussion on the predictive ability of the derived calibration model is provided.

C. Materials and Methods

1) Samples

Analytical grade anhydrous D-(+)-glucose, D-(−)-fructose, and sucrose in powder form were purchased from Sigma–Aldrich (Sigma, St. Louis, MO, USA). Standard stock solutions were prepared from each appropriate sugar dissolved in distilled water. For each sugar, 5 solutions containing 10, 30, 60, 100, and 150 mg/mL were prepared. Three technical repetitions were made for each concentration for every analyte (n=15) used (glucose, fructose and sucrose). Distilled water was used as reference for subsequent differential processing. For the validation phase, four commercial soft drinks note for their sugar concentration were employed: Sprite, CocaCola, and Fanta produced by The Coca-Cola Company (Atlanta, USA), and Pepsi by PepsiCo (Purchase, USA), obtained from a local store. According to their label, the sugar content of these soft drink in the Italian market is respectively 19 mg/mL, 106 mg/mL, 118 mg/mL, and 109 mg/mL. Three technical repetitions were made for each beverage sample. Every sample, pipetted in optical cuvettes, was measured 5 times. All samples were measured at room temperature, which was kept under control (26.5 – 27.5°C).

2) *Instrumentation*

The spectral data were collected in transmittance mode using the benchtop instrument VIS-NIR Lambda 45 Spectrometer from PerkinElmer (Waltham, USA) and a custom setup composed of: an infrared light source with range 360-2400 nm, fiber optics, and a CUV-ALL-UV Cuvette Holder, all obtained from Ocean Optics (Dunedin, USA), and a NIR micro-spectrometer MS-series C11708MA obtained from Hamamatsu Photonics (Shizuoka, JP). The benchtop instrument has an operative range of 190–1100 nm with a resolution spanning from 0.5 to 4 nm. It uses a light-dispersion device, a monochromator, to produce a narrow bandwidth of monochromatic light which is then conveyed toward a sample. The amount of monochromatic light transmitted through the sample is registered by a detector and converted to an electrical signal. The mini-spectrometer is based on MOEMS (micro-opto-electro-mechanical-system) technology. It comprises an input slit, which is the opening for receiving the light to be detected; a grating, which separates the incident light into each wavelength, reflected at different diffraction angle; an image sensor, which converts the spectrum of light into electrical signal. In particular, it integrates a Complementary Metal-Oxide Semiconductor

(CMOS) sensor with wavelength sensing range 640-1050 nm and spectral resolution of max 20 nm. In order to couple the spectrometer entrance slit to the fiber optics, it was designed and realized a customized optical interface, composed of several optical components, including a SMA 905 adapter, an aspheric collimator, and two cylindrical lens from Thorlabs (Newton, USA). The overall interface was put in optical axis with the spectrometer slit by means of cages and assembly rods. The complete experimental setup is shown in Figure 12.

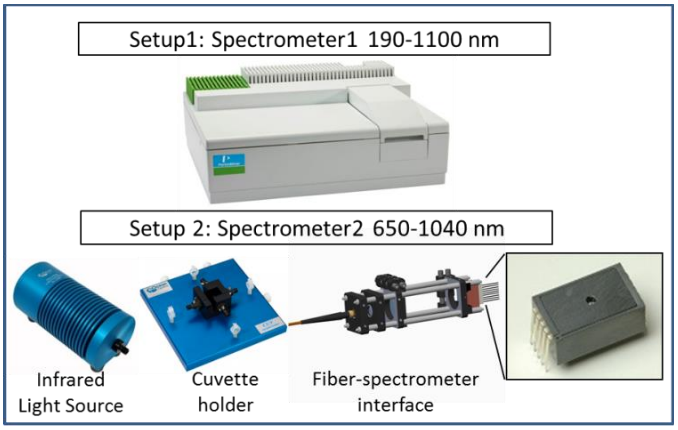

Figure 12. Experimental setup schematics.

3) Experimental design

A benchtop instrument and a custom setup were used to acquire spectra in transmittance mode of aqueous solutions of four sugars at different concentrations, for the calibration phase, and of three commercial soft drinks used as real samples for validating the performance of the implemented model. Each sample, previously prepared, were pipetted inside optical cuvettes of 10 mm path length. Regarding the PerkinElmer instrument, after selecting the software settings of 1.2 nm resolution, 5 cycles repetition and 2880 nm/min measurement speed, sample spectra were then acquired and all the phases described were managed using the UWLab software. Hamamatsu proprietary software was used to acquire spectra with the mini-spectrometer, using the following setting parameters: 1200 msec as integration time, 5 measurements as repetition and low gain. An optical diffuser was placed on the light source fiber end side, to avoid saturation of the sensor, and a cuvette cover was used to screen off the ambient light. The benchtop instrument directly output the absorption spectrum, while for the mini-spectrometer was necessary to perform a proper instruments calibration, consisting of the preliminary acquisitions of the light source reference spectrum I_0(total transmission) and the dark spectrum $\mathrm{D}(\lambda)$ (zero transmission). This phase was necessary to define the signal dynamics that could be expressed with that configuration. After acquiring the transmission spectrum of a sample $\mathrm{I}(\lambda)$, the related absorption spectrum $\mathrm{A}(\lambda)$was calculated with the following expression:

$$\mathrm{A}(\lambda) = -\log_{10}\left(\frac{\mathrm{I}(\lambda) - \mathrm{D}(\lambda)}{\mathrm{I}_0(\lambda) - \mathrm{D}(\lambda)}\right)$$

All the acquisitions were performed at ambient temperature between 26.5 and 27.5 °C.

4) Methodology

The acquired spectra contained both chemical information and noise. Firstly, the average for every concentration was calculated. Then spectra were pre-processed to filter out the noise and finally a second derivative processing was implemented, to highlight the absorption peak inside noisy spectra. Indeed, peak resolution substantially improves adopting a second derivative of absorbance spectra [70]. In particular, a Norris–Williams second-order derivative algorithm was performed (six-point smoothing and gap size of six), which comprised the following steps:

i) a first smoothing filter to reduce noise and keep useful variation;

ii) a subtraction of distilled water spectrum from the original spectra, to further enhance the spectral effect due to the organic compounds [71];

iii) a second smoothing filter computed on the results from previous subtraction step;

iv) a second derivative computed as finite difference, using the moving window:$(s_{i+g} - s_i) - (s_i - s_{i-g}) = s_{i-g} - 2s_i + s_{i+g},$ where $s = (s_1, s_2, \dots, s_k)$ was the data resulting from the previous steps and g the width of the considered window[72].

Eventually, the characteristic wavelengths in the spectra were highlighted selecting the informative range 945-972 nm for the dataset obtained with Setup1 and 945-984 nm for the dataset obtained with Setup2. Mild spectral signal shift is always introduced when measuring a sample with different instruments, mainly in x-axis adjustment (wavelength accuracy) and y-axis response (absorbance). This is due to the constructional differences between system, which is mainly reflected in different spectral resolutions. Moreover, such differences are accumulated building prediction model by multivariate methods [73]. At this stage, the cropped spectra were fitted with a 6th degree polynomial, the lowest order that best interpolated the data, and the curves integral was evaluated in the two ranges to calculate the area subtended by the peak. The obtained data were then used to build a linear regression model with the area as abscissa and the correspondent concentrations as ordinate. Two linear prediction models were obtained for each setup. Next step was the repetition of the same pre-processing, using the dataset collected from the four real beverages samples. The obtained area values were used to feed the calibration lines to predict the concentration values. The Mean Square Error of Prediction was then evaluated using the predicted values and the real concentrations reported on the drinks labels. The whole processing was implemented in Matlab version R2015a from MathWorks (Natick, USA).

D. Results

The results from the pre-treatment phase conducted on dataset 1 and dataset 2 are depicted respectively in Figure 13 and Figure 14. The derivative pre-processing highlighted the wavelengths sensitive to the sugar concentration variation, i.e. 945 - 972 nm in the case of Setup1 and 945 - 984 nm in the case of Setup2, which were also in agreement with the findings from the loadings analysis performed on principal components (Figure 15). The remaining less-informative parts of the spectra were cut out before proceeding to the next steps.

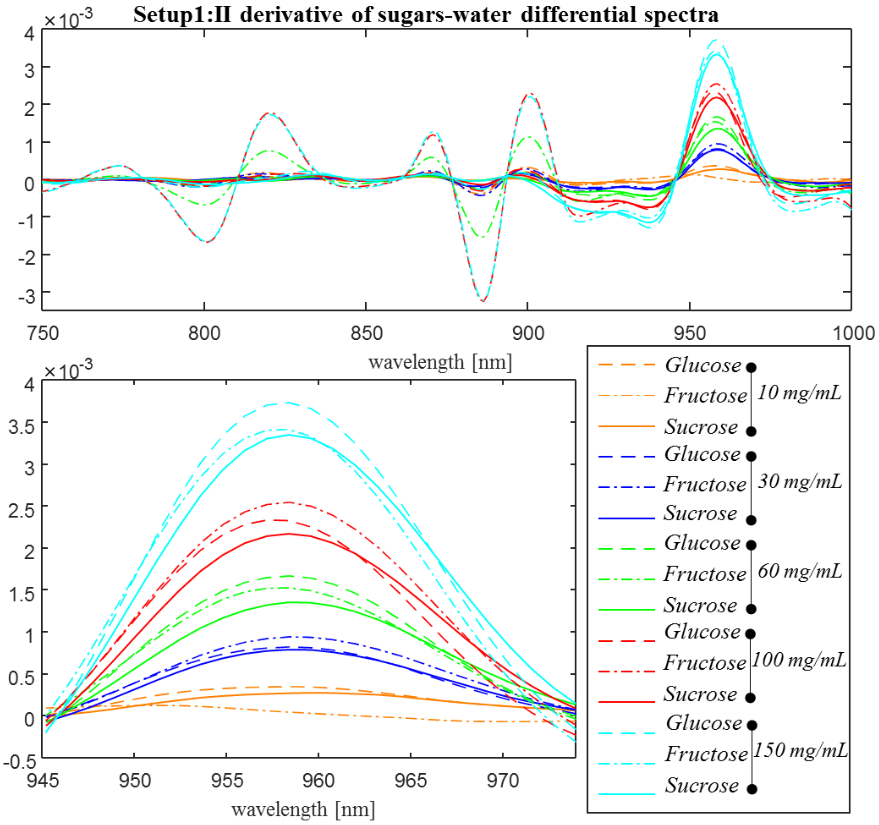

Figure 13. Result of pre-treatment implemented on dataset acquired with Setup1, which highlights the sensitive spectral range 945-972 nm.

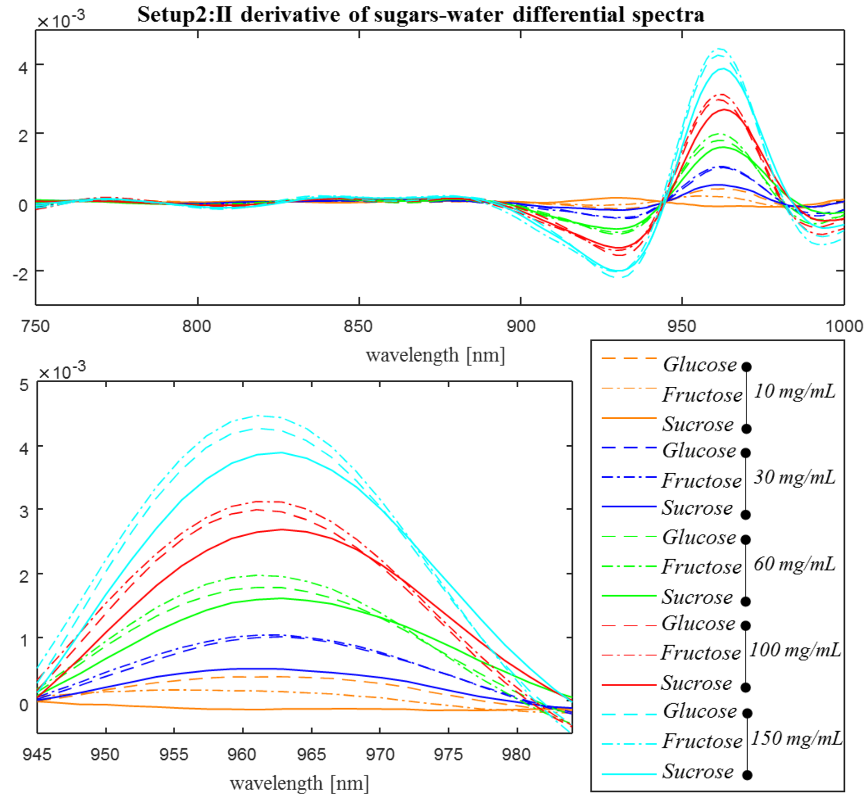

Figure 14. Result of pre-treatment implemented on dataset acquired with Setup2, which highlights the sensitive spectral range 945-984 nm.

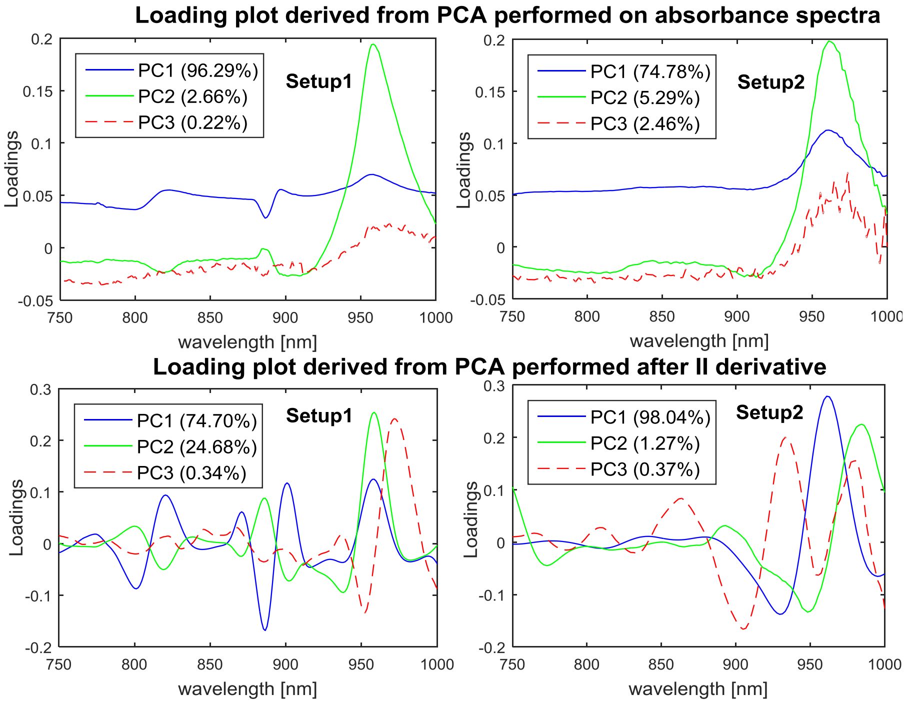

Figure 15. Loadings analysis performed on the first three principal components highlights the wavelenghts most sensitive to variation of sugar content related to Setup1 and Setup2.

1) *Calibration phase*

For each concentration-related partial spectrum, the corresponding area subtended under the peak was calculated, and these values were used to build the calibration line. The results from this step are depicted in Figure 16 for dataset 1 and Figure 17 for dataset 2. R^2 = 0.9850 (the linear fit of the concentration data explains 99.85% of its variance) evaluated from the calibration obtained with dataset 1, while R^2 = 0.9816 evaluated from the calibration obtained with dataset 2.

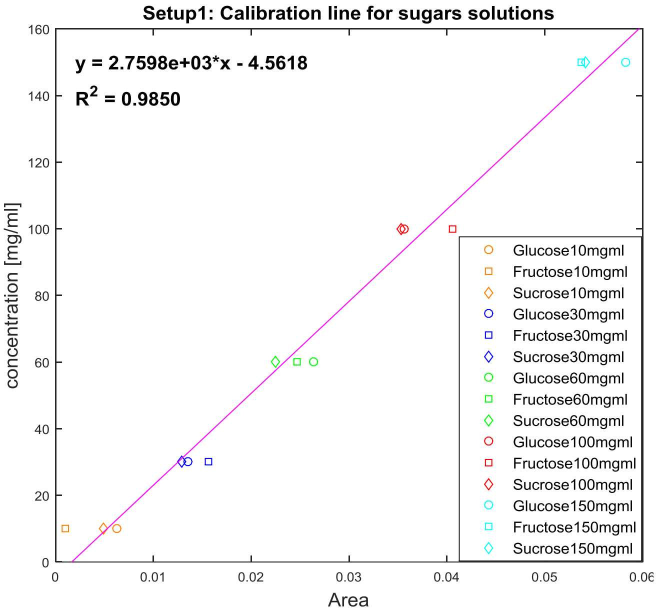

Figure 16. Calibration curve calculated from data acquired with Setup1. (available range of calibration data 10 – 150 mg/ml).

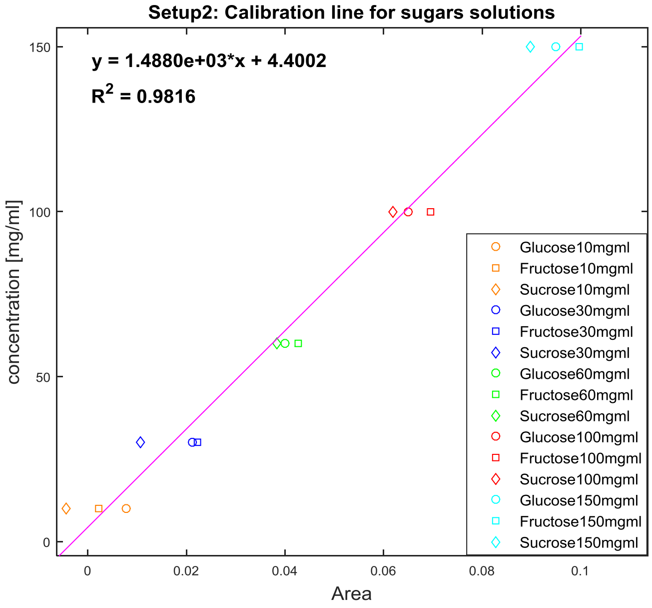

Figure 17. Calibration curve calculated from data acquired with Setup2. (available range of calibration data 10 – 150 mg/ml).

2) *Validation phase*

Four commercial beverages were used to validate the obtained calibration models. Same processing performed on sugar solutions dataset was implemented on soft drinks dataset. The subsequent areas were given as input to the previously developed models to predict the sugar concentration inside beverages. These values were compared to the tabulated values on the drinks labels and a related Root Mean Square Error of Prediction (RMSEP) was calculated as evaluation index of prediction performance, using the form:

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(y[i] - \hat{y}[i])^2}{n}}$$

where $y[i]$ are the real values and $\hat{y}[i]$ the predicted values. Results from this phase are shown in Figure 18.

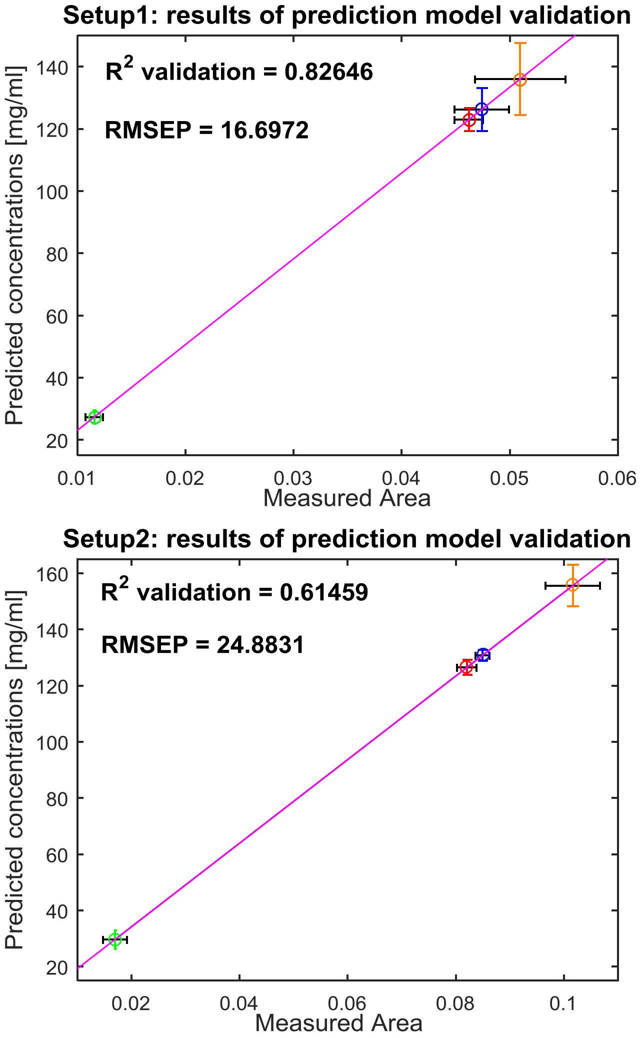

Figure 18. Results of Setup1 and Setup2 prediction models validation with 12 real samples (available range of validation data 19 – 118 mg/ml).

E. Discussions

Although in calibration the two models showed fairly similar and very high coefficients of determination, in validation, they showed quite different performances. Only the model based on Setup1 obtained predicted values close to real values. The model based on Setup2 proved to be significantly less accurate, which can be explained by the mini-spectrometer instrumental characteristics of lower resolution, compared to the benchtop instrument, and by the limit in the choice of acquisition time, conditioned by the saturation of the sensor. Therefore, a different instrumental strategy must be found to allow an increase in integration time without incurring saturation of the sensor. Furthermore, the significant difference between the calibration determination coefficient and the validation coefficient in both models can be explained by a substantial difference between the nature of the calibration set and the nature of the test set. Two possible resolutions of this limit can be implemented. The first consists in the building of a calibration based on a set composed of incremental addition of sugar to, and serial dilutions of, the nominal sugar content of various beverage samples, so as to cover a wide range. Indeed, similar works found in literature were successful in measuring sugar content in different food liquid matrices by using the same mixture divided in different samples for the calibration, cross-validation and test phases. This condition allowed researchers in [59] to reach very high prediction results by using a benchtop FT-NIR instrument in transmittance mode in the range 800-2400 nm for the quantification of glucose, fructose and sucrose in bayberry juice. Good prediction results (R^2 = 0.84) were also achieved by Marrubini et al. [69] in determining sugar content in different plant milk samples with a benchtop instrument but using wavelengths in the MIR range as selected variables. Very good prediction peformances (R^2 = 0.92) were also obtained in [67] with a MLR model for the determination of reducing sugar content during grape ripening. The second option consists of a sample pre-treatment step for the carbonated drinks in ultrasonic bath, so as to completely remove the carbon dioxide component whose presence may obstruct the light beam when crossing the optical path of the sample. This is what is done in [74] by Ilaslan et al., in which glucose, fructose, and sucrose were quantified in commercial soft drinks by using Raman spectroscopy obtaining as coefficient of determination (R^2) values of 0.913, 0.998 and 0.993 for validation, respectively.

F. Conclusions

In this chapter, a multi-platform analytical method was proposed to predict sugar content in beverages, through the realization of a regression model based on the most sensitive range in the near-infrared absorbance spectra regarding change in sugar concentration. Aqueous solutions of glucose, fructose, and sucrose at different concentrations were prepared and absorption spectra in the NIR range were collected at the same temperature using a benchtop instrument and a custom setup. Spectral datasets were pre-processed and treated with second-order derivative analysis. The most sensitive wavelengths for sugar content were highlighted selecting the informative range 945-972 nm from analysis of dataset acquired with the benchtop instrument and 945-984 nm from analysis of dataset acquired with the mini-spectrometer. In these ranges the capacity of separating absorbance spectrum belonging to different concentration was maximized. Thereafter, a regression algorithm was calculated from the cropped spectra, establishing the relationship between the instrument response and analytes concentrations. Validation has been then performed with four commercial soft drinks containing different known sugar content, which spectral dataset were acquired with the same operative conditions used for sugar solutions samples.

The achieved results, validated with real samples, displayed an acceptable interoperability, having tested the efficacy on two different spectral acquiring platforms. As a next step, we will expand the test set using other kind of common used beverages, like fruit juices, to strengthen the validation of the models. We will also investigate the realization and subsequent validation of a model built on dataset acquired at different temperatures.

Being an easy adaptable method, since calibration of the system is performed according to the desired application, the proposed methodology can be exploited for realizing concentration-based calibration models for different analytes dissolved in water, which absorbance characteristics have overtones in the NIR range.

III. PORTABLE OPTICAL PLATFORM FOR SUGAR CONTENT MEASUREMENT

In this Chapter, exploiting the wavelengths in the NIR range most sensitive to sugar concentration in liquids, the design, development and testing of a LED-based optical platform for sugar content measurement in beverages is presented.

A. State of the art

Many epidemiological studies reported that sugary drinks significantly increase the risk of developing Type 2 diabetes [75]–[78]. Additionally, frequent consumption of sugars has been associated with a greater risk of dental caries [79]. Reference Intake (RI) establishes the maximum recommended nutrient intake and recently, the term RI has replaced by 'Guideline Daily Amount' (GDA). Beside the terminology, the principles behind how these values are determined remain the same. The major difference is that GDAs existed for men, women and children; instead, there is only one set of RIs for an average adult, based on the requirements for an average female with no special dietary requirements and an assumed daily energy intake of 2000 kcal. According to Regulation (EU) 1169/2011 by European legislation on the provision of food information to consumers [80], which is based on scientific advice from the European Food Safety Authority (EFSA), the RI for sugars is set to 90 g/day. According to the American Heart Association, and considering that one teaspoon of added sugars has the same amount of total sugars as 1 teaspoon (4 g) of table sugar (sucrose), men should limit added sugars to 9 teaspoons, or 150 calories, per day and women should eat no more than 6 teaspoons, or 100 calories, from added sugars each day [81]. Most US adults report consuming more added sugar than recommended for a healthy diet and are seeking ways to cut back sugar intake [82]. Results from survey conducted in [83] showed that just 4% of respondents correctly classified 10 or more ingredients from a presented list of 13 items, while 65% of participants were unaware of the WHO guidelines for sugar intake [84]. These studies highlighted a serious and widespread problem of an unwitting over-intake of sugar, far above the recommended assumption. This unawareness gap could be bridged by a sensor solution.

The new generation of mobile sensing approaches offers significant advantages over traditional platforms in terms of test speed, control, low-cost, ease-of-operation, and data management, and requires minimal equipment and user involvement. The marriage of novel sensing technologies with cell-phones enables the development of powerful lab-on-smartphone platforms for many important applications, especially food analysis [85]. Among the different sensing strategies, mobile spectrometry has a disruptive potential, due the intrinsic property of being a non-invasive, rapid, reagent-free technique, whose features can be even more enhanced by coupling with advanced data analysis and machine learning techniques [86].

In order to support users or patients, simplified, easy to use and low-cost mobile diagnostic devices for real-time measurements of dietary sugar are needed. current state of the art of mini-spectrometers allows the development of portable and lower cost solutions for food analysis. For example, Researchers in [87] developed a portable scanner, composed of UV LED and spectrometer, to be used in combination with smartphone to measure fruit ripeness. While substantial progress has recently been made in the miniaturization of near-infrared (NIR) spectrometers, there remains continued interest in pushing the technology development toward even smaller, simplified, and lower cost analysers. The potential of these instruments to revolutionize on-site applications can be realized only if the reduction in size does not compromise performances. Variable selection methods have been proposed in various studies in order to exploit only few wavelengths in the absorbance spectra, and thus LEDs light rather than using broadband lamps. Researchers in [88] proposed a LED-based system at four specific wavelengths for the rapid evaluation of fruit and vegetable quality. An optoelectronic system with LEDs at three identified wavelengths was developed and tested in [89] for measuring ethanol concentration in fermenting grape must. Researchers in [90] have used five near infrared light emitting diodes (LEDs) and a photodiode to measure the intensity of the reflected light from pineapples, to classify the internal quality using neural network. Understanding of light-emitting diode lamp behaviour is essential to support the use of these devices as illumination sources in portable optical analysers [91]. A comparison between the cited simplified LED-based platforms and the system that will be described in this Chapter, with respect to the introduced innovative points, is presented in Table 4.

Table 4. Comparison table between the state of the art of LED-based food analyzers and the system proposed in this work.
+ Figure of merit. / Not provided.

	Das et al [87]	**Civelli et al [88]**	**Jiménez-Márquez et al [89]**	**Rateni**
Measurement mode	Reflectance	Reflectance	Transmittanc e	Transmittance
Versatility	-	++	-	++
Modularity	-	++	-	++
Precision	/	+	++	-
Low-cost	+	/	-	++

Following the rapid development of chemometrics research, NIR spectroscopy has been more and more applied to the analysis of liquid foods [92]. The major benefit of working with liquid samples is the high repeatability of measurement that can be reached using transmission mode, due to low scattering characteristics of samples which are contained in cuvette with fixed optical path length [93]. To the best of authors knowledge there is a lack in literature regarding simplified LED-based optical sensing devices for sugar content measurement in liquids. This work aims at the design of a rapid, low-cost, portable optical analyser for sugar content measurement in beverages. The proposed system represents a simplified and far lower cost version of a spectrophotometer operating in transmittance mode. The concept design of the optical platform will be described, followed by a list of hardware opto-electronic components. Afterward, the calibration by means of model solutions will be illustrated, followed by the validation by means of real samples consisting of three soft drinks with different sugar content. Eventually, a discussion on the predictive ability of the derived calibration model is provided.

B. Materials and Methods

1) Reference Instrument

The spectral data were collected in transmittance mode a custom setup composed of: an infrared light source with range 360-2400 nm, fiber optics, and a CUV-ALL-UV Cuvette Holder, all obtained from Ocean Optics (Dunedin, USA), and a NIR micro-spectrometer MS-series C11708MA obtained from Hamamatsu Photonics (Shizuoka, JP). The mini-spectrometer is based on MOEMS (micro-opto-electro-mechanical-system) technology. It comprises an input slit, which is the opening for receiving the light to be detected; a grating, which separates the incident light into each wavelength, reflected at different diffraction angle; an image sensor, which converts the spectrum of light into electrical signal. In particular, it integrates a CMOS sensor with wavelength sensing range 640-1050 nm and spectral resolution of max 20 nm. In order to couple the spectrometer entrance slit to the fiber optics, it was designed and realized a customized optical interface, composed of several optical components, including a SMA 905 adapter, an aspheric collimator, and two cylindrical lens from Thorlabs (Newton, USA). The overall interface was put in optical axis with the spectrometer slit by means of cages and assembly rods. The complete experimental setup is shown in Figure 19.

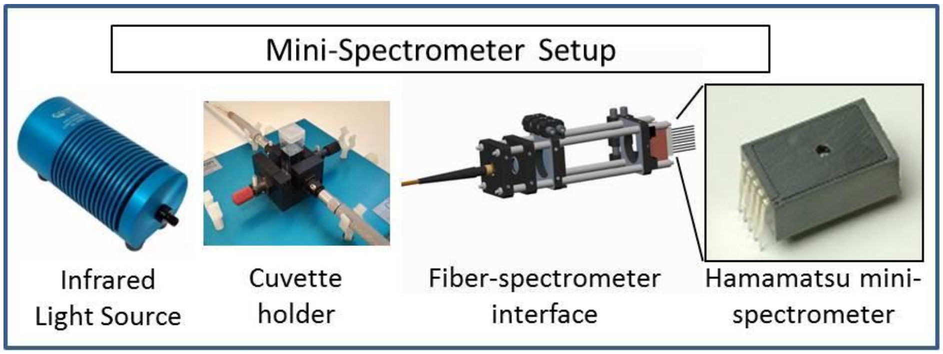

Figure 19. Experimental setup schematics of the reference instrument.

2) *Concept design of the optical platform*

The system consists of a simplified, miniaturized and lower cost version of a spectroscopic platform in transmittance mode, which in particular uses LED lamps as light source, to tune the system at selected wavelengths, based on spectral bands selected in previous studies. The choice of using LEDs allowed to obtain a very versatile and modular concept, which can be opportunely adapted for different applications and different kinds of sample matrices, while keeping its main architecture. The LEDs were not directly directed towards the sample, in order to avoid in-homogeneities of sample irradiation. An integrating sphere with diameter of 50 mm was rather used to collect and spatially integrate the radiant flux from LEDs, spreading the incoming light by multiple diffuse reflections over the entire sphere inner surface. The LED lamps were arranged inside the integrating sphere around the perimeter of a port opening, with diameter of 5 mm, which was being used as large area source that features uniform radiance. The LEDs and the port opening were located in the same hemisphere and in particular, the LEDs were placed inside tilted openings carved through the hemisphere around the central port. The port opening was positioned in the maximum horizontal circumference of the sphere in a central position with respect to the LEDs. The sphere internal surface was brushed with Titanium White acrylic paint Liquitex® (Cincinnati, USA) to serve as reflectance coating. Light outgoing from the sphere through the opening was channelled to the cuvette holder, designed to accommodate optical cuvette of 10 mm path length. Finally, transmitted light was conveyed to a photo-detector, opportunely lodged inside a dedicated slot, with the sensitive area facing the sample and positioned at the height of the optical axis. CAD design of the described system is provided in Fig. 2, while in Fig. 3 a schematics of the optical part of the system is depicted. The overall frame was 3D printed and the device dimensions were 62x62x84 mm.

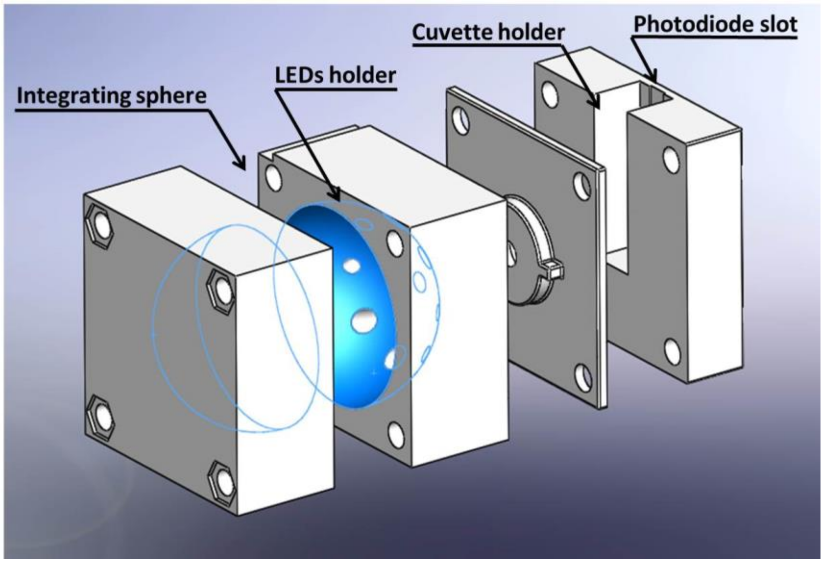

Figure 20. CAD design of the 3D printed platform frame.

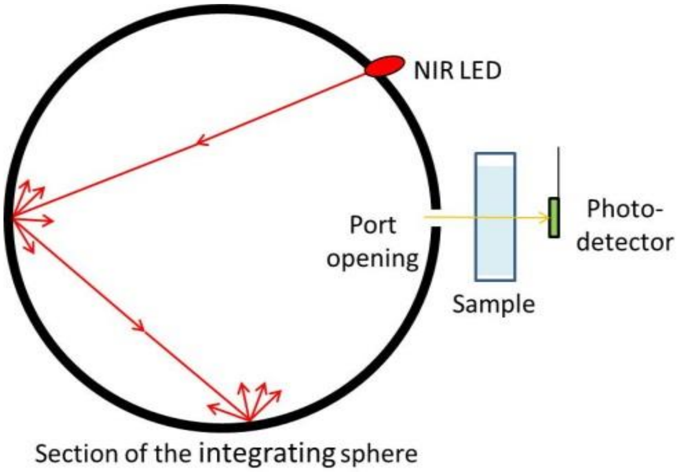

Figure 21. Schematics of the optical part of the system.

3) *System Hardware*

The LEDs were chosen at the wavelengths that most closely matched the theoretical choice, according to the availability on the market. Four LEDs with peak wavelengths at 950, 960, 970, and 980 nm were purchased from Roithner Lasertechnik (Vienna, Austria) and Vishay Intertechnology (Malvern, USA). Having chosen a forward current of 10 mA to increase components lifespan, a current limiting resistance of 370 Ohm was connected to each LED anode, calculated by using the following formula:

$$R = \frac{V_f - V_s}{I_f}$$

where V_f and I_f are respectively the Forward Current and Forward Voltage (extracted from datasheet) and V_s is the Supply Voltage, which in the case of USB supply is 5 V. In Table 4 the LEDs characteristics are summarized.

Table 5. LEDs electric characteristics and the relative current limiting resistor.

	Forward Current	Forward Voltage	R @ 5V supply
TSUSS200 950	10 mA	1.3V	370 Ohm
ELD-960-525	10 mA	1.35V	370 Ohm
LED970-06	10 mA	1.3V	370 Ohm
LED980-03	10 mA	1.25V	370 Ohm

Regarding the electronics aspects, a sequential activation of each LED channel was preferred to a simultaneous one, to avoid inter-channels interferences. An Si photodiode, purchased from Luna Optoelectronics (Camarillo, USA), with spectral response spanning from 400 to 1100 nm, was aligned with the integrating sphere port opening, and lodged at the end of the measurement optical axis, to collect and measure the optical radiation transmitted from the sample. A double layer printed circuit board (PCB) was designed with the open-source KiCAD software and made with a circuit board plotter ProtoMat S63 from LPKF (Garbsen, Germany) for conveniently supplying and controlling the LEDs and for conditioning the photodiode signals. In Figure 22 the overall architecture of the PCB is depicted. The photocurrents delivered by the photodiode was converted into voltage signals by means of a transimpedance amplifiers. The gain resistance was empirically selected in order to in order to spread the signal on the whole dynamic provided by the power supply. The supporting frame was designed with SolidWorks and made of polylactic acid (PLA) using a Ultimaker 2+ 3D Printer (Geldermalsen, The Netherlands). This frame was designed to hold in a compact form the optoelectronic components in precise positions for optimized absorbance measurements in transmittance mode, with the sample holder lodged between the light source and the detector, and aligning the photodiode along the optical axis of the sphere port opening. The sample holder, provided with a lid to screen out ambient light, was designed to perfectly fit an optical cuvette of 1 cm path-length.

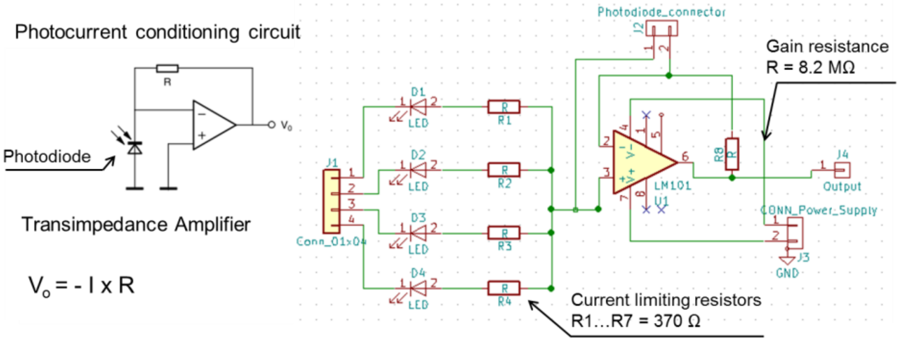

Figure 22. Schematics of the photocurrent conditioning circuit and overall architecture of printed circuit board.

4) *Data Acquisition*

The output from the photocurrent amplifier was connected to an analog input pin of an Arduino-compatibile Elegoo UNO R3 board (Shenzhen, China), based on ATmega328P microcontroller from Microchip (Chandler, USA), which was used to acquire the signal. After digital conversion of the signal operated by means of a 10-bit ADC peripheral, the signal was sent to a PC using serial communications via USB with a baud rate of 9600 baud. The microcontroller run a memory pre-loaded routine, which individually and sequentially controlled the powering of each LED, according to a specific sequence. The acquisition routine can be schematically described as follows:

i) the signal is acquired ten times and then the average is calculated;
ii) the digital levels are translated in voltage signals;
iii) a IIR digital filter stage using a feedback term is implemented to obtain a stable output with respect to the fluctuation of the LEDs;

5) *Sample Preparation*

Analytical grade anhydrous D-(+)-glucose, D-(−)-fructose, and sucrose in powder form were purchased from Sigma–Aldrich (Sigma, St. Louis, MO, USA). Standard stock solutions were prepared from each appropriate sugar dissolved in distilled water. For each sugar, 5 solutions containing 10, 30, 60, 100, and 150 mg/mL were prepared. Three technical repetitions were made for each concentration for every analyte (n=15) used (glucose, fructose and sucrose). Distilled water was used as reference for subsequent differential processing. For the validation phase, commercial soft drinks note for their sugar concentration were employed: Sprite (19 mg/mL), CocaCola (106 mg/mL), RedBull (110 mg/mL), and Fanta (118 mg/mL), obtained from a local store. Three technical repetitions were made for each beverage sample. Every sample, pipetted in optical cuvettes, was measured 5 times. All samples were analysed in a fixed range of temperature (26.5 – 27.5 °C).

6) *Experimental design*

The implemented platform and the reference instrument were used to acquire spectra in transmittance mode of aqueous solutions of three sugars at different concentrations, for the calibration phase, and of three commercial soft drinks used as real samples, for validating the performance of the implemented models. All the acquisitions were performed at ambient temperature between 26.5 and 27.5 °C. Each sample, previously prepared, were pipetted inside optical cuvettes of 10 mm path length. Hamamatsu proprietary software was used to acquire spectra with the mini-spectrometer, using the following setting parameters: 1200 ms as integration time, 5 measurements as repetition and low gain. An optical diffuser was placed on the light source fiber end side, to avoid saturation of the sensor, and a cuvette cover was used to screen off the ambient light. An initial proper instrument calibration, consisting of the preliminary acquisitions of the light source reference spectrum $I_0(\lambda)$ (total transmission) and the dark signal D (zero transmission), was conducted on both the instruments. This phase was necessary to define the signal dynamics that could be expressed with that configuration. After acquiring the transmission spectrum of a sample $I(\lambda)$, the related absorption spectrum $A(\lambda)$was calculated with the following expression:

$$A(\lambda) = -\log_{10}\left(\frac{I(\lambda) - D}{I_0(\lambda) - D}\right)$$

The Arduino IDE was used to launch the pre-compiled acquisition routine and serial communication via USB port at baud rate of 9600 baud was used to receive data on the laptop, for subsequent post-processing. After a waiting of one minute, to allow the IIR filter stabilization, the data were saved into a text file, in separated channels for each LED and with a size of 50 values each, ready to be analysed.

7) *Post-processing*

Since the number of observations was larger than the number of variables, data acquired with the optical platform were processed to build a multivariate calibration model by using Multiple Linear Regression (MLR), where the dependent variable is modeled as a linear combination of the independent variables and the regression coefficients are estimated with the least squares criterion [94]. The obtained MLR model using the four independent variables had the following form:

$$\mathrm{y} = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_4 x_4$$

where y represents the estimated property, the sugar concentration), x_i the wavelength variables, and b_i the regression coefficients of the model. Dataset acquired with mini-spectrometer were firstly pre-processed to filter out the noise and highlight the absorption peak inside noisy spectra using a Norris-William second-order derivative algorithm [95], eventually selecting the sensitive range 940-980 nm. Since in this case, the number of variables was larger than the number of samples, Partial Least Squares Regression (PLSR) method was chosen, which is by far the most widely used technique in quantitative analytical chemistry [96]. The models implemented respectively with MLR and PLSR methods were then validated with real samples with different known sugar concentration. Coefficient of Determination (R-Squared) related to calibration and validation were used as indices for evaluating performances of the two implemented regression models. The whole processing was implemented in Matlab version R2015a from MathWorks (Natick, USA).

C. Results and Discussion

The resulting prototype of the designed portable optical platform is shown in Figure 23.

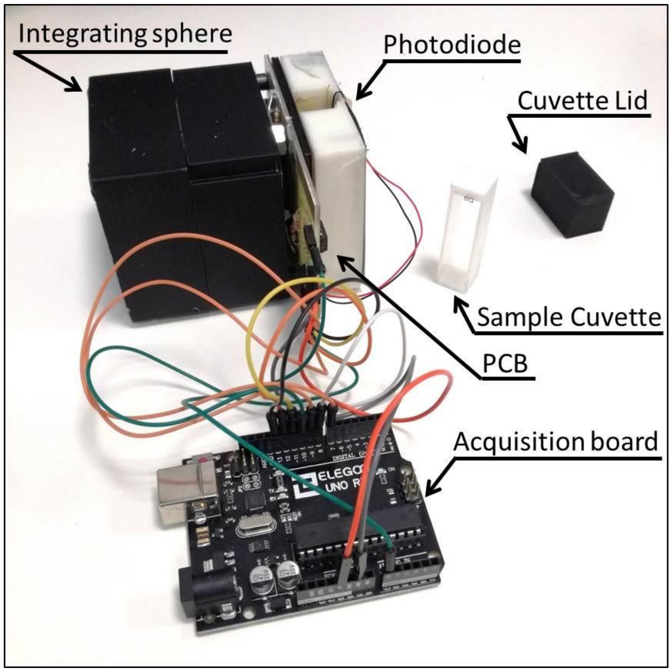

Figure 23. The final prototype connected to the acquisition board.

Calibration phase

A MLR algorithm was implemented to build the prediction model using data acquired with the optical platform, obtaining a Coefficient of Determination of $R_{cal}^2 = 0.90$, evaluated from the obtained calibration model. A PLSR algorithm was implemented to build the prediction model using data acquired with the reference spectrometer, obtaining a Coefficient of Determination $R_{cal}^2 = 0.96$, evaluated from the obtained calibration model.

Validation phase

Three commercial soft drinks were used to validate the obtained calibration models. Same processing performed on sugar solutions dataset was implemented on soft drinks dataset. The validation of the MLR model led to a Coefficient of Determination of $R_{val}^2 = 0.5$, while the validation of the PLSR model led to a Coefficient of Determination of $R_{val}^2 = 0.91$. The overall results from calibration and validation phases are shown in Figure 24.

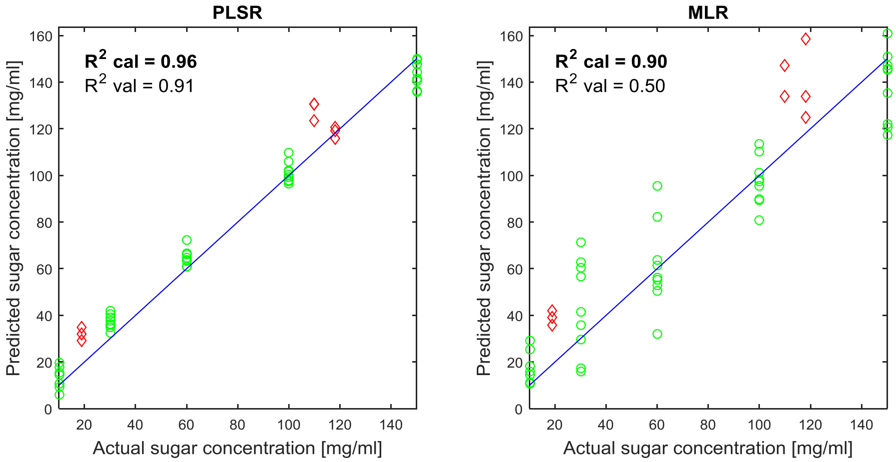

Figure 24. Comparison of the actual and predicted sugar content, with sugar aqueous solutions used for calibration(green circles) and soft drinks used for validation(red rombi).

D. Discussions

In calibration the two models showed good coefficient of determinations, with MLR model that proved to be slightly less accurate than the PLSR model, according to what has already been highlighted in the work by Giovenzana et al. [97]. Differently, in validation, while PLSR performed quite well, MLR showed a significant loss in performance, which is highlighted by the difference between the calibration determination coefficient and the validation coefficient. This is probably due to the high sensitivity of the system to the soft drinks carbonation, whose presence may obstruct the light beam when crossing the optical path of the sample. As described in [74] by Ilaslan et al., a sample pre-treatment phase implemented for carbonated drinks sample, consisting in a ultrasonic bath, can be used to completely remove the carbon dioxide component. Regarding chemometrics improvement, data pre-treatment by using baseline correction, detrend or derivative processing may be implemented before operating the regression, in order to better underline the signal related to sugar content with respect to the remaining non-informative and noisy data.

E. Conclusions

In this work, the design, implementation and testing of a portable LED-based optical platform for sugar content measurement in beverages have been described, according to prior studies conducted by the author, where sensitive wavelengths range in the near-infrared absorbance spectrum were found by means of optimized chemometrics processing. These results were exploited in this work to conceive an optoelectronic system which used as light source LEDs with peak emission wavelengths selected in this range, according to the closest match available on the market. The developed platform is a simplified, lower cost and portable version of a spectroscopic device in transmittance mode, which uses a Si photodiode as detector instead of a much more expensive spectrometer. The concept design and the hardware composition of the device have been described. An experimental setup have been conceived to calibrate the device using aqueous solutions of glucose, fructose, and sucrose at different concentrations. After building the prediction model for sugar content, using a MLR algorithm, initial data has been collected to evaluate the model using commercial soft drinks containing different known sugar content, which discrete spectral dataset were acquired with the same operative conditions used for sugar solutions samples. The performances of the developed model were compared with those of a PLSR model, built upon spectra acquired using a reference spectroscopic instrument. As a next step, we will investigate hardware and software strategies to enhance platform performances, then the realization and subsequent validation of a model built on dataset acquired at different temperatures will be investigated. Eventually, the adoption of a better MCU architecture for signal processing will be valuated to provide the platform with an embedded unit to process and visualize real-time data results of sugar content in liquid samples.

Being the designed system a modular and versatile platform for what concern the selection of LEDs, according to the specific application, the proposed concept can be exploited for realizing prediction models for different target analytes dissolved in water.

IV. NOVEL INDUSTRIAL APPLICATION OF NIR SPECTROSCOPY

In this Chapter the proof of concept of an automated packaging sorting system based on near infrared reflectance spectroscopy is described.

A. Introduction

Traditional waste sorting methods in the recycling industry mainly depend on the processed material since metals, glass, plastics, paper, and woods have very different physical properties. Most advanced sorting techniques, like indirect sorting, introduce advanced sensors and techniques including Laser Spectroscopy, X-rays, Optical Sorting, Hyper-Spectral Imaging and Reflectance Spectroscopy [98]. In the indirect sorting, sensors are used for detecting recyclable materials in the bulk input waste followed by segregation using various actuators or robotic systems as in [99]. The indirect sorting using Near-Infrared Reflectance (NIR) spectroscopy allows faster and safer identification of different materials respect to other methods such as X-Ray and Laser spectroscopy, while is cheaper than using expensive hyperspectral cameras. It is successfully used for chemical analysis, materials classification and quality increase of recycled products. Indeed, high purity recycled materials ensure optimum technical and environmental performances, while impurities or contaminations can cause a reduction in quality. For this reason it is today more and more investigated for advanced sorting. Recently, the use of NIR spectrometers has been investigated in the industrial field to meet specific standards determined by industrial applications, such as characterizing exhausted lubricating oils [100,101], in the separation of plastic packaging materials [102], to separate recycled building and demolition inerts [103], and in the separation of composite wood-plastic materials[104].

Taking in account for the state of the art, the identification of mineral oil contamination on cardboard and plastic packaging is still a research challenge, with significant implication in recycling and industry 4.0. The proposed solution could positively contribute to the definition of new automated methods for advanced and high quality sorting.

B. Materials And Methods

1) Samples

Traditional packaging from automotive industry were selected including four different type of cardboard boxes. Boxes had three different background colours (light brown, dark brown and white) and black and blue inks printed inscriptions. The different backgrounds and the coloured inks of prints have been introduced as confounding factors both for the NIR sensors and for the classification algorithm. Then the cardboard boxes were greased using engine lubricant oil and used as contaminated samples.

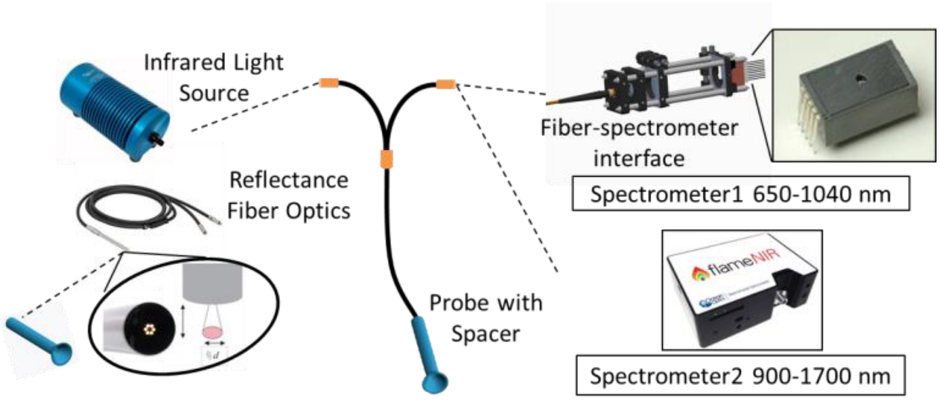

Figure 25. Experimental setup schematics.

2) Instrumentation

Two different spectrometer sensors were tested:

- FlameNIR from Ocean Optics (Dunedin, USA). The sensor, featuring an uncooled InGaAs detector with wavelength range 950-1650 nm, has a spectral resolution of max 10 nm;
- MS-series C11708MA from Hamamatsu Photonics (Shizuoka, JP). The mini-spectrometer integrates a CMOS sensor with wavelength sensing range 640-1050 nm and spectral resolution of max 20 nm. In order to provide SMA 905 connector to the sensor, it was coupled with a customized optical interface, realized by means of several optical components, including a SMA adapter, an aspheric collimator, and two cylindrical lens from Thorlabs (Newton, USA).

A modular experimental setup was assembled as in Figure 25, consisting of a high-power infrared light source with range 360-2400 nm from Ocean Optics (Dunedin, USA), connected to the measuring system through a RP23 fiber optic reflection probe from Thorlabs (Newton, USA).

3) *Experimental design*

The two spectrometers were used to acquire NIR reflectances spectra directly on the sample surfaces. The probe was placed perpendicularly, 8 mm above 10 surfaces from different samples: (i) four different clean cardboard samples, (ii) black and blue prints on cardboards, and (iii) three contaminated cardboard surfaces. For each surface, 100 different acquisitions were saved, for a total of 1000 observations (Table 6). The same integration time (465 μs) was set on the two spectrometers.

Table 6. Summary of samples used in the experimental phase

Type	**Material type (repetitions)**	**Observations**
Clean	light brown cardboard (200) dark brown cardboard (200) white cardboard (100) black-ink prints (100) blue-ink prints (100)	700
Contaminated	Oil on light brown cardboard (200) Oil on dark brown cardboard (100)	300

4) *Methodology*

The observed 1000 spectra contained both chemical information and noise. Samples were initially pre-processed to filter out the noise (stray light, light scattering, detector non-linearities, etc), according to the following steps:

1. outlier rejection: using the Hampel method [105] to improve the classification ability of multivariate methods;

2. Standardization: using the Standard Normal Variate (SNV) transformation;
3. weighting: samples were weighted to compress the dynamics in the range [0,1].

The Leave-Multiple-Out (LeaveMOut) cross-validation method was implemented leaving out the 10%, 30% and 50% of the dataset to validate the proposed classification system. The dataset was randomly divided into training and test sets. Then the compositional information were extracted performing the Principal Component Analysis (PCA) on the training-set [106]. Eventually, the samples were projected on the principal components space. A Support Vector Machine (SVM) algorithm was trained on the projected training-set, to distinguish between contaminated and non-contaminated cardboards. After the training, the PCA was applied to the test-set, and the trained SVM classified the PCA outputs. The LeaveMOut method was repeated 1000 times on the dataset, to achieve statistical significance. The PCA, the training of the SVM and the LeaveMOut method were implemented in Matlab version R2012a from MathWorks (Natick, USA).

C. *Results*

The proposed system successfully distinguished and classified contaminated cardboard samples from non-contaminated ones, even in the presence of confounding factors, including black and blue ink prints. The classification accuracy was up to 99.68%, performing the spectral analysis in the range 950-1650 nm. Using the 640-1050 nm NIR bandwidth, the accuracy was up to 99.64%. Nevertheless, the latter can be considered a cost-effective solution for low-cost applications. The worst classification performance was achieved leaving 50% of the samplings for the training of the SVM and using the remaining 50% of samples for testing. In this case the maximum number of classification mistakes were 79 and 59 respectively for the Hamamatsu and the Ocean Optics spectrometers. Using the 90% of the samples for the training and the 10% for the testing, the maximum classification error decreased up to 34 and 33 respectively for the Hamamatsu and the Ocean Optics sensors. The classification accuracy thus mainly depended on the training-set size, and in a residual manner on the spectrometer sensing range. The results of the LeaveMOut validation process are reported in Table 7.

Table 7. Classification accuracy of the proposed system using the Ocean Optics and the Hamamatsu sensors

Spectrometer sensing range	**950-1650 nm**			**640-1050 nm**		
Training set [% of sample size]	**10%**	**30%**	**50%**	**10%**	**30%**	**50%**
Max classification errors	33	59	44	34	40	79
Mean classification errors	3.21	6.40	10.44	3.60	7.48	12.30
Mean classification accuracy	99.68%	99.36%	98.96%	99.64%	99.25%	98.77%

D. Conclusions

The proposed work highlights the opportunity to perform an accurate classification of oil-contaminated cardboard from non-contaminated one, to improve the purity of the sorting output and the quality of the next recycling process. The achieved results positively impact on to the research of new sorting methods, by analysing the combination of synthetic oil and cardboard in the NIR spectrum. Furthermore, the achieved classification accuracy fits the previous works on the classification/sorting of contaminated materials. Indeed the work proposed in [104], claimed an output purity of the 97.1% in the sorting of wood samples, respect to wood-plastic samples, and a purity of the 98.9 % in the sorting of wood respect to wood contaminated with preservatives. This preliminary work demonstrates that both the sensing ranges 950-1650 nm and 640-1050 nm can be profitably used. Future activities will concern the analysis of more cardboard samples and the introduction of more confounding factors, including water drops, different types of inks and prints, applied plastic tape, foils or sheets. Furthermore, different pre-processing techniques will be investigated, as well as the opportunity to implement different classification techniques, including K-Means and neural networks.

IV. THESIS CONCLUSIONS AND FUTURE WORKS

This doctoral thesis has dealt with the study and development of non-invasive optical analytical methodologies, with food analysis and nutrition monitoring as main target applications. After an in-depth review of the state of art of new technologies enabling portable food analysis, the near-infrared spectroscopy methodology has been chosen for its great potential for providing a user-friendly, scanning on-the-go scenario. It indeed allows peculiar rapid multi-component non-invasive and non-destructive analysis, and is a greener alternative to reagent-based analytical technique; no need for reagents and limited waste after the analysis. However, being a non-specific method, it must be associated with multivariate statistical and chemometric tools for spectral dataset analysis to extract the desired chemical information. Indeed, calibration and tuning of the systems are performed according to the desired application.
As widely discussed, in a view to simplify hardware and decrease apparatus cost, critical design criteria are represented by the proper selections of the wavelength range for source and detector, and of the measurement setup.

The research novelties introduced with this work were the proposition of a multi-platform optimized analytical method based on the most informative wavelength range in the NIR region and the design and development of a portable low-cost LED-based optical platform, both specifically implemented for the quantitative measurement of sugar content in aqueous solutions and beverages. In particular, the choice of this application was motivated by the widespread reported problem of unwitting over-intake of sugar, far above the recommended assumption, and also by the discomfort of diabetes patients which have to manually calculate the meal glycaemic index. This work proposes a step towards a possible consumer mobile diet companion for sugar tracking in beverages.

A multi-platform analytical method able to predict sugar content in beverages was developed, through the realization of regression models based on the most sensitive range in the near-infrared absorbance spectra regarding change in sugar concentration. These results were then profitably exploited to develop an optical platform for sugar content measurement in beverages based on LEDs as light sources and silicon photodiode as detector, using the previous analytical findings as a cue for design and as a reference for comparison of results. The developed platform was a simplified, lower cost and portable version of a spectroscopic device in transmittance mode, which uses a photodiode as detector instead of a much more expensive spectrometer. In addition, the selection of the near infrared optical window and silicon photodiodes allowed to dramatically decrease product cost, compared to the infrared optical window and InGaAs photodiodes.

Being the designed system a modular and versatile platform for what concern the selection of LEDs, according to the specific application, the proposed concept can be exploited for realizing prediction models for different target analytes dissolved in water.

Calibration and validation of the platform have been implemented, highlighting the current hardware, software, and calibration limitations of the developed system, and proposing related improvement strategies. In particular, current study limitations are represented by the low efficiency of MLR prediction model implemented for the optical platform, highlighted by the difference between the calibration determination coefficient and the validation coefficient. This was probably due to the high sensitivity of the system to the soft drinks carbonation, whose presence may obstruct the light beam when crossing the optical path of the sample. As proposed in [74] by Ilaslan et al., a sample pre-treatment phase implemented for carbonated drinks sample, consisting in a ultrasonic bath, can be used to completely remove the carbon dioxide component. Regarding chemometrics improvement, data pre-treatment by using baseline correction, detrend or derivative processing may be implemented before operating the regression, in order to better underline the signal related to sugar content with respect to the remaining non-informative and noisy data.

Regarding future works, the test set will be expanded using other kind of common used beverages, like fruit juices, to strengthen the validation of the models. The realization and subsequent validation of a model built on dataset acquired at different temperatures will be also investigated, in order to approach a real usage scenario.

In a view to highlight the wide range of applicability of portable spectroscopy, a further application has been proposed for the field of intelligent industrial automation. Indeed, the Industry 4.0 paradigm requires new technologies and methods not only to improve the profitability and the quality of the industrial production and products, but also new strategies to reduce the social and environmental impact of the production process. In particular, near infrared reflectance spectroscopy has been proposed and implemented to empower a robotic arm with material classification capabilities, for in-line automated sorting of waste.

In conclusion, the results accomplished in this Phd work demonstrated that the near infrared spectroscopy methodology can be successfully exploited to develop portable solutions for food analysis and nutrition monitoring. Furthermore, it has been shown that the range of application of this tecnology can also be extended to the very different sector of industrial automation, demonstrating the multi-disciplinarity of the subject matter.

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