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Optical measurements and pattern-recognition techniques for identifying the characteristics of beer and distinguishing Belgian beers

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ABSTRACT

A miscellaneous assortment of 86 beers was characterized using non-destructive, rapid and reagent-free optical measurements. Diffuse-light absorption spectroscopy performed in the visible and near-infrared bands with the use of optical fiber spectrometers was tested innovatively to gather turbidity-free spectroscopic information. Furthermore, conventional turbidity and refractive index measurements were added in order to complete the optical characterization. The scattering-free near-infrared spectra provided a novel and straightforward turbidity-free assessment of the alcoholic strength. The entire optical data set was then processed by means of multivariate analysis in a search for a beer grouping in accordance with the characteristics and identity of each type. The results indicated that optical technologies could be successfully used for beer differentiating between several classes of beers. Moreover, since half the beers were typical Belgian beers, multivariate processing of the optical data was also applied in order to achieve a differentiation of the Belgian beers as compared with all the others, thus demonstrating a potential method for authenticating the country of production.

1. Introduction

Beer originates from prehistoric times [1]. Nowadays, with an average consumption of about 80 l per person in 2009 [2,3], beer is the third most often consumed drink worldwide, after water and tea, and the first most widely consumed alcoholic beverage. Indeed, since it consists of 93% water, beer is a refreshing, enjoyable, thirst-quenching long drink with a relatively low alcoholic strength and glycemic load which brings pleasure to and instigates social interaction among many people. In addition to water, beer is made from wholesome raw materials: malted barley, other cereals, hops, and yeast. As in any natural food, many healthy ingredients can be identified in beer [4], including antioxidants [5] – mainly polyphenols [6], essential vitamins – particularly B vitamins [7,8], and minerals. While underage and heavy drinking have harmful effects and leads to chronic diseases, recent studies have reported that a responsible light-to-moderate consumption of beer by healthy adults has many beneficial effects, including a reduction in the risks of cardiovascular diseases, osteoporosis and diabetes [9]. Moreover, beer is the only significant dietary source of hops, which are not only responsible for the bitter taste and provide preservative agents, but are also a unique source of isohumolones, which can reduce hyperglycemia in subjects with prediabetes [10].

Indeed, beer is an undistilled fermented beverage, in which a source of starch consisting of malted barley and wheat is converted by means of hot water into a sugary liquid which undergoes a fermentation process triggered by the addition of yeast. Many types of beers are brewed depending on the type of producer, which range from multinational companies to small producers, or else brewpubs or regional micro-breweries that produce with a limited amount of artisan-made beers [11,12].

Beers are differentiated mainly according to their visual appearance and their fermentation process. The visual appearance of beer depends on both its color and its turbidity. This is not only because scattering reduces the transparency of beer, but also because the suspended particles themselves can contribute to light absorption. Both these factors are heavily influenced by the brewing method. Thermal treatment can be applied to different extents in order to accelerate the drying of the malt, and this results in darker, more reddish, beers [13]. Suspended sediments due to yeast residuals can be found in top-fermented beers, which are often unfiltered or bottle-conditioned. The use of malted or unmalted wheat also influences the beer's color. At times, other colorants, such as caramel, are added in order to "adjust" the color to the desired shade.

Depending on the yeast used and the fermentation temperature, three main beer categories can be identified: top-fermented,

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bottom-fermented, and naturally-fermented. Top-fermented beers, which are also called "Ale", are produced by adding *saccharomyces cerevisiae* yeast in the 15–20 °C temperature range, and offer a fruity-flavored aroma. Bottom-fermented beers, which are also called "Lager", are produced by adding *saccharomyces uvarum* (or pastorianus) at cooler temperatures, namely between 7 and 12 °C: the lower temperature inhibits the production of esters and other by-products, thus producing a cleaner-tasting beer. Naturally-fermented beers, which are produced mainly in Belgium, where are commonly called "Lambic", make use of wild rather than cultivated yeasts. The entirely natural process guarantees an unusual aroma and sourness.

Every recipe and production method, provides the beer in question with a distinctive quality and taste. Indeed, the quality differentiation of beers, as of many other foodstuffs, is a marketing requisite that exists in order to satisfy consumers who have become more critical, more demanding and more fragmented in their food choices, especially in developed countries. It has been demonstrated that competing on the basis of price alone is no longer the most effective business strategy. A holistic approach that satisfies a sense of good mood and positive emotions is a more modern, attractive, and consumer-oriented tactic, which equates quality with all the desirable properties that a product is perceived to have [14]. In front of a crowded shelf in a supermarket, or inside a small niche-shop, the consumers' cognitive mechanisms are driven by attractive indicators, especially by three main extrinsic cues: brand, country of origin, and quality label [15,16]. With particular reference to the beer market, it has been demonstrated that brand identity [17,18], the country of production [19–21], and a label with information about the manufacturing process [22], provided differentiating and added-value concepts linked to sensory properties.

Optical methods, especially optical spectroscopy, provide a modern and effective means for non-destructive food analyses. In fact, in addition to non-destructive testings, they represented a "*green*" approach to sustainable analytics, since they are considered to be user-friendly, general, and moderate-cost technologies [23]. Optical methods measure the sample "*as it is*", without using reagents, functionalization, or chemical treatments. Since no chemical reagents are involved there are no discharges to recycle. Moreover, avoiding treatments leads to a safer handling of samples which is helpful also in the case of non-trained operators. The potential lack of selectivity and sensitivity can be compensated for by using mathematical algorithms – the so-called system intelligence. Typically, chemometrics has been considered to be a robust and popular approach for processing optical data; in particular, for more than 40 years, it has demonstrated effectiveness in all kinds of spectroscopic applications [24,25].

Many spectroscopic studies have been dedicated to beer-quality applications, always combined with a chemometric treatment of optical data. Fluorescence measurements have been used for monitoring beer quality during storage [26], for analyzing vitamins [27], as well as for assessing the content of nutraceutic factors such as riboflavin and aromatic amino acids [28], in addition to the renowned "resveratrol" antioxidant compound [29]. Near-infrared spectroscopy alone, and the combination of mid- and near-infrared spectroscopy have been extensively employed for quantifying important quality parameters of beers, such as alcoholic content, and original and real extracts [30–34]. The most recent works on optical methods for beer-quality assessment have shown how to confirm the brand identity of a famous Belgian beer by using near-infrared transflectance spectroscopy [35], and how to discriminate beers of the same brand, even if brewed in different factories, using spectroscopic data which have been fused with other sensory data from e-tongues [36].

In order to meet the requirements of product differentiation and authentication, this paper presents an experiment which makes use of non-destructive, rapid and reagent-free optical measurements for the purpose of recognizing beer varieties. A wide collection of beers was considered; these were produced in different countries using all fermentation methods. Unusual and more conventional setups for optical measurements were used to analyse the entire collection. Diffuse-light absorption spectroscopy, performed by means of an integrating sphere in the visible and near-infrared bands and using optical fiber spectrometers, proved to be capable of providing scattering-free absorption measurements – that is, without having to take into consideration of the natural turbidity of the beer, which could impair traditional absorption spectroscopy measurements. In addition to these unusual absorption spectroscopy measurements, other physical parameters such as turbidity and the refractive index were measured using conventional optical instruments, in order to complete the optical characterization of the beer collection. Intuitively speaking, while diffuse-light absorption spectroscopy provided information on color (the visible band) and alcoholic strength (the near-infrared band), the turbidimetry and refractometry were related to the intrinsic sample turbidity and to Brix, respectively. Indeed, Brix provides the sugar content of an aqueous solution. It is usually measured by means of refractometry, so as to keep track of the degree of fermentation [37].

A straightforward prediction of the alcoholic strength was obtained by means of the scattering-free near-infrared spectra, thus demonstrating a novel approach which can be used online during beer production. The scattering-free visible and near infrared spectra, together with the turbidity and refractive index values were then combined for the first time to create a data matrix, which was processed by means of multivariate analysis. This novel method was capable of grouping the beer collection according to the individual fermentation method (Lager, Ale, Lambic), and color (Golden, Dark). These groups reflect the individual identities of the various beers, thus indicating that optical technologies can be successfully used for beer differentiation among several classes. Moreover, since half of the beers were typical Belgian beers, multivariate processing of the optical data was also applied in order to achieve a classification of the Belgian beers with respect to all the others, thus demonstrating a method for authenticating the country of production.

2. Experimental

2.1. The beer collection

The beer collection used in this experiment consisted of 86 beer samples, each of a different brand. 50 beers were produced in Belgium, while the others came from other countries, such as Italy, Germany, Denmark, England, The Netherlands, Japan, The Czech Republic, Cuba, and Mexico, as listed in Table 1. These were top-fermented (Golden Ale, Dark Ale, Weiss), bottom-fermented (Lager, Doppelbock, Mexican), and spontaneously-fermented (Golden and Cherry Lambic) beers, with different colors, and alcoholic strengths in the 0.5–11% vol range.

Only one bottle for each beer type was available. In order to mitigate experimental uncertainties, each measurement was performed three times, and the average was then taken. All experimental data reported refer to the average values.

2.2. Diffuse-light absorption spectroscopy in the visible and near-infrared bands

Diffuse-light absorption spectroscopy makes use of an integrating sphere that contains the sample being tested [38–40]. The

Table 1
The beer collection.

Country	Code	Brand	Class	Alcohol	Turbidity (NTU)	Refractive Index
Belgium	VB01	Oude Geuze Boon 2007	Golden Lambic	7.0%	190.0	1.342
Belgium	VB02	Oude Geuze Boon 2008	Golden Lambic	7.0%	195.0	1.342
Belgium	VB03	Den Herberg Blond	Golden Ale	5.5%	149.0	1.344
Belgium	VB04	Den Herberg Tarwe	Weiss	5.0%	59.4	1.343
Belgium	VB05	Gueuze Girardin	Golden Lambic	5.0%	135.0	1.341
Belgium	VB06	Grimbergen	Golden Ale	6.7%	1.5	1.342
Belgium	VB07	Hoegaarden	Weiss	4.9%	113.0	1.341
Belgium	VB08	Palm	Golden Ale	5.4%	3.2	1.341
Belgium	VB09	Satan Gold	Golden Ale	8.0%	48.7	1.343
Belgium	VB10	Taras Boulba	Golden Ale	4.5%	125.0	1.341
Belgium	VB11	Bersalis Tripel	Golden Ale	9.5%	10.2	1.347
Belgium	VB12	Kriek Lindemans	Cherry Lambic	3.5%	24.4	1.349
Belgium	VB13	Affligem	Golden Ale	6.8%	19.4	1.343
Belgium	VB14	Kessel	Golden Ale	7.5%	20.9	1.341
Belgium	VB15	Servais	Golden Ale	5.5%	67.7	1.342
Belgium	VB16	Hof ten Dormaal	Golden Ale	8.0%	712.0	1.344
Belgium	VB17	Oude Kriek Boon	Cherry Lambic	6.5%	147.0	1.343
Belgium	L01	Bink	Golden Ale	5.5%	8.4	1.343
Belgium	L02	Ter Dolen	Golden Ale	6.1%	19.6	1.343
Belgium	L03	Jessenhofke Tripel	Golden Ale	8.0%	41.0	1.342
Belgium	L04	Sint Gummarus Tripel	Golden Ale	8.3%	19.6	1.343
Belgium	L05	De Chokier	Dark Ale	7.0%	163.0	1.344
Belgium	L06	Cristal 1928	Lager	5.8%	3.5	1.340
Belgium	L07	Maya (Jessenhofke)	Golden Ale	6.0%	90.0	1.342
Belgium	WV01	Balthazar	Dark Ale	8.9%	392.0	1.344
Belgium	WV02	Keyte Triple	Golden Ale	7.7%	35.2	1.344
Belgium	WV03	Harlekijn	Golden Ale	6.0%	17.3	1.340
Belgium	WV04	Alternatief	Golden Ale	5.0%	407.0	1.340
Belgium	WV05	Bockor Pils	Lager	5.2%	7.9	1.340
Belgium	WV06	Blauw Export (Bockor)	Lager	5.2%	7.4	1.340
Belgium	WV07	Brugse Zot	Golden Ale	6.0%	7.9	1.342
Belgium	OV01	Gentse Strop	Golden Ale	6.9%	25.2	1.342
Belgium	OV02	Troubadour Blond	Golden Ale	6.5%	28.3	1.344
Belgium	OV04	Vicaris Tripel	Golden Ale	8.5%	50.5	1.343
Belgium	OV05	Valeir Extra	Golden Ale	6.5%	83.0	1.342
Belgium	OV06	De Graal	Golden Ale	8.0%	35.8	1.339
Belgium	A01	De Koninck Tripel	Golden Ale	8.0%	13.6	1.344
Belgium	A02	Bienvenue de Montaigue Tripel	Golden Ale	7.0%	688.0	1.343
Belgium	A03	Lucifer	Golden Ale	8.0%	10.2	1.342
Belgium	A04	Gouden Carolus	Dark Ale	8.5%	17.5	1.347
Belgium	A05	Karmeliet Tripel	Golden Ale	8.4%	22.9	1.347
Belgium	A06	Malheur 6	Golden Ale	6.0%	77.3	1.340
Belgium	A07	Westmalle Tripel	Golden Ale	9.5%	22.0	1.343
Belgium	A08	Duvel	Golden Ale	8.5%	54.4	1.343
Belgium	H01	Magus Belgo	Golden Ale	6.6%	61.1	1.344
Belgium	H02	Belgoo Luppoo	Golden Ale	6.5%	26.1	1.343
Belgium	H03	Moinette Blond	Golden Ale	8.5%	14.7	1.341
Belgium	H04	Ciney Blond	Golden Ale	7.0%	5.2	1.342
Belgium	N01	Leffe Blond	Golden Ale	6.6%	1.5	1.344
Belgium	LU01	La Chouffe Blond	Golden Ale	8.0%	17.7	1.343
Italy	I1	Moretti Baffo d'Oro	Lager	4.8%	3.4	1.340
Italy	I2	Pedavena	Lager	5.0%	1.6	1.341
Italy	I3	Ichnusa	Lager	4.7%	2.2	1.340
Italy	I5	Poggio del Farro	Golden Ale	5.5%	86.9	1.343
Italy	I6	Libra	Lager	5.8%	5.7	1.341
Italy	I7	Peroni	Lager	4.7%	1.6	1.339
Italy	I8	Tourtel	Lager	0.5%	1.3	1.340
Italy	I9	Moretti Rossa	Doppelbock	7.2%	2.6	1.345
Italy	I10	La Matta Ambrata	Dark Ale	6.5%	8.2	1.345
Italy	I11	Glencoe	Dark Ale	6.5%	32.4	1.345
Italy	I12	La Monella	Golden Ale	5.0%	20.7	1.342
Italy	I13	La Biscara	Golden Ale	7.2%	67.5	1.345
Italy	I14	Cosimo	Dark Ale	5.1%	237.0	1.341
Italy	I15	Caterina	Golden Ale	5.1%	86.0	1.341
Italy	I16	Menabrea & Figli	Lager	4.8%	2.0	1.340
Germany	G1	EKU 28	Doppelbock	11.0%	3.4	1.351
Germany	G2	HB Original	Lager	5.1%	1.5	1.340
Germany	G3	DAB	Lager	5.0%	1.6	1.341
Germany	G4	Warsteiner	Lager	4.8%	2.5	1.340
Germany	G5	Beck's	Lager	5.0%	1.7	1.341
Germany	G6	Franziskaner Weisse	Weiss	5.0%	89.6	1.341
Germany	G7	Riedenburger Weisse	Weiss	5.4%	37.4	1.342
Denmark	D1	Ceres TOP Pilsener	Lager	4.6%	1.2	1.339
Denmark	D2	Tuborg	Lager	5.0%	1.2	1.340
Denmark	D3	Ceres Strong Ale	Golden Ale	7.7%	1.4	1.343
Denmark	D4	Ceres Red Erik	Dark Ale	6.5%	1.0	1.341
Denmark	D5	Ceres Old 9	Lager	9.1%	1.1	1.341
Japan	J1	Asahi	Lager	5.0%	1.7	1.340
Japan	J2	Kinin Ichiban	Lager	5.0%	2.0	1.339
Japan	J3	Sapporo	Lager	5.0%	1.7	1.341
England	E1	Ruddles County	Dark Ale	4.7%	1.4	1.341
England	E2	Tennent's	Golden Ale	9.0%	1.9	1.345
Netherland	N1	Bavaria 8.6	Lager	7.9%	4.7	1.342
Czech	Z1	Budejovicky Budvar	Lager	5.0%	1.0	1.341
Cuba	C1	Mayabe	Lager	4.0%	1.7	1.337
Mexico	M1	Corona	Mexican	4.6%	2.1	1.341

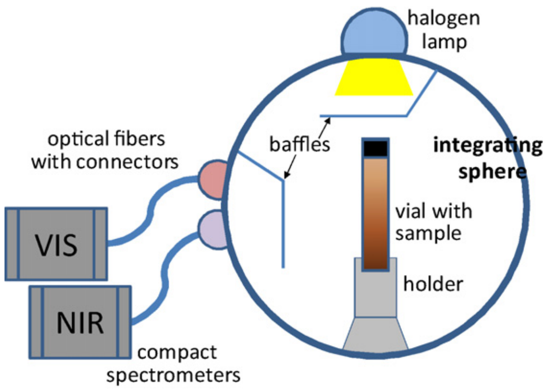

Fig. 1. Schematic diagram of the setup for diffuse-light absorption spectroscopy using optical fiber technology.

source and the detector are butt-coupled onto the sphere. Almost all the light shining on the sphere surface is reflected diffusely, and the introduction of an absorbing sample in the cavity causes a reduction in the radiance in the sphere which is independent of other scattering effects induced by suspended particles in the sample. This technique has been successfully used for a scattering-free characterization of different liquids, such as lubricant oils and extra virgin olive oils [41,42].

The setup used for beer measurements is shown in Fig. 1. A vial containing 32 ml of beer sample was inserted in a 22 cm diameter custom integrating sphere which was equipped with a 100 W halogen lamp and with two fiber optic spectrometers for detecting the visible and near-infrared diffuse-light spectra. Fig. 2 shows the practical implementation of this setup [43]. The integrating sphere was closed, and a sliding device made it possible to insert and to extract the glass vial. The spectrometer for the visible band was the USB4000 model from OceanOptics [44], and the spectrometer for the near-infrared band was the SuperGamut model from BaySpec Inc. [45]. The measured bands and resolutions were: 450–800 nm and 0.2 nm for the visible, and 900–1200 nm, and 1.7 nm for the near-infrared, respectively. Optical fibers with 50-μm and 200-μm core diameters were used for the visible and near-infrared spectroscopic measurements, respectively, so as to equalize the light intensity in the different bands. Prior to each beer measurement, the spectra of a vial filled with distilled water were recorded for referencing. Fig. 3 shows the results of the diffuse-light absorption spectroscopy measurements. Fig. 3-left shows the spectra in the 450–800 nm range, while Fig. 3-right shows the first-derivative spectra in the 900–1200 nm range.

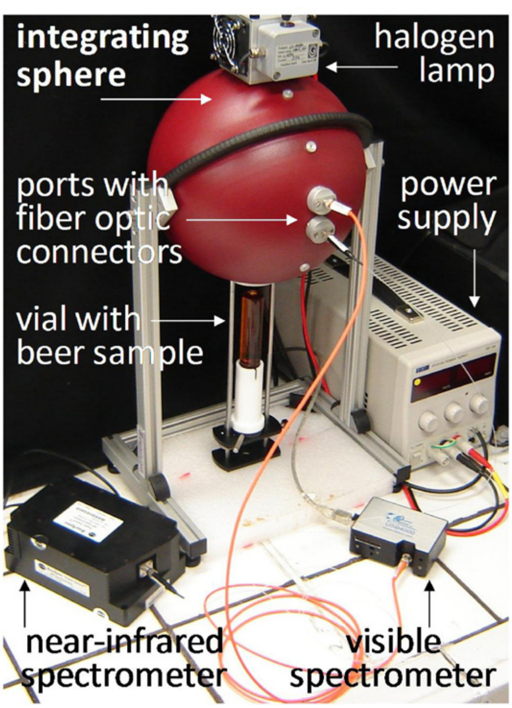

Fig. 2. Practical implementation of the experimental setup for diffuse-light absorption spectroscopy.

The visible spectra distinctly separate the golden beers from red ones, and show the entire palette of color shades. As expected from the scattering-free measurements, all golden beer spectra are in the lower part of the plot, notwithstanding the turbidity which is fairly high for several of the Belgian beers (i.e. Hof ten Dormaal and Bienvenue de Montaigue Tripel). First-derivative spectra instead of simple absorption are shown in order to better highlight the absorption peak spread, which appeared limited in the near-infrared range. As expected, since the near-infrared range provides information on alcoholic strength, low-alcohol beers such as the Lagers show smaller absorption peaks than those of more alcoholic beers such as Doppelbocks and Dark Ales. The noisy spectrum close to the 0-level refers to the Tourtel, which is nearly analcoholic (0.5% only); consequently, the similarities between the beer and the water spectra cause a poor signal-to-noise ratio.

In order to compress the spectroscopic information, the tri-stimulus values defined by the CIE1931 color space were computed [46,47]. The tri-stimulus coordinates were obtained from the Irradiance spectrum of the cavity which, in an integrating sphere, is proportional to the radiance spectrum. This calculation was made for both the sample and for a reference vial filled with pure water. Then, in order to compensate for source fluctuations, the difference between the two chromatic vectors was calculated and the components of the difference vector were indicated by the letters X, Y, and Z. Thus, these variables express the color-distance between the beer sample and the reference vial.

Fig. 4 shows the result of the colorimetric processing. Fig. 4-left shows the behavior of the luminance, Y, as a function of the beer type. The beers were correctly positioned according to their own luminance: the darker, which have a lower luminance (Cherry Lambic, Dark Ale, and Doppelbock) are positioned at the plot top (i.e. farther from the reference); the lighter, which have a higher luminance (Golden Ale, Golden Lambic, and Lager) are positioned at the plot bottom (i.e. closer to the reference). Fig. 4-right shows the x–z plot in which x and z were normalized variables that were used to separate the purely chromatic information from the brightness information:

$$x = \frac{X}{X+Y+Z}$$

$$z = \frac{Z}{X+Y+Z}$$

The beer collection was correctly represented according to the beer color by means of a U-shape behavior, with the Golden Ale beers at the vertex.

2.3. Turbidity and refractive index measurements

Turbidity measurements were performed by means of a commercially available compact turbidimeter (Hach, model 2100-P). The intensities of 0°-transmitted and 90°-scattered light were measured, and ratioed in order to provide the sample turbidity in Nephelometric Turbidity Units (NTU). The instrument worked in the 0–1000 NTU range with ±2% accuracy. Fig. 5 shows the results of the turbidity measurements. The distribution of these turbidity values was heavily right-skewed (skewness = 3.8). This is

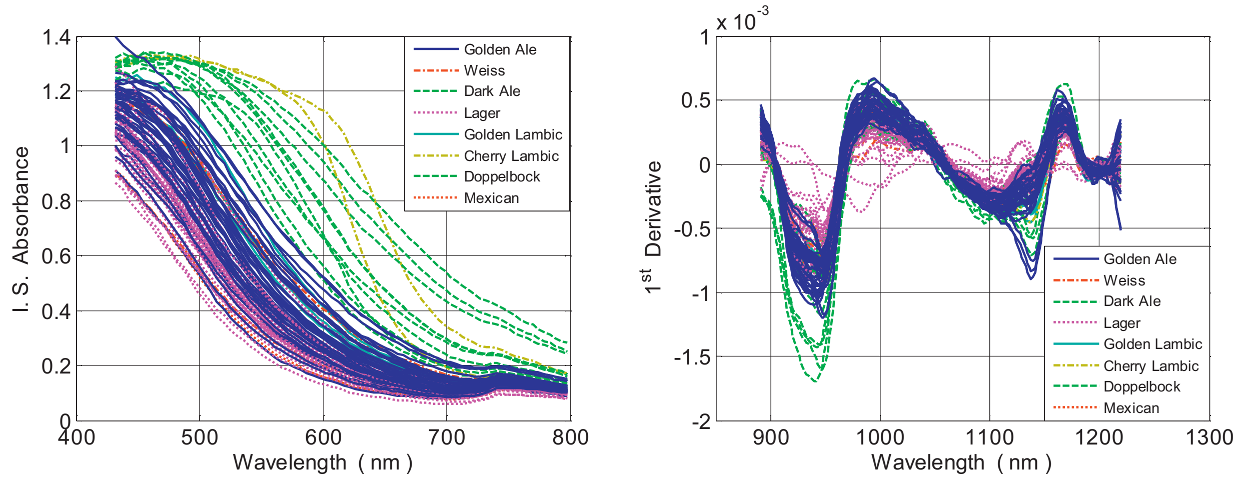

Fig. 3. Diffuse-light absorption spectra of the beer collection – Left: visible spectra; Right: first-derivative near-infrared spectra.

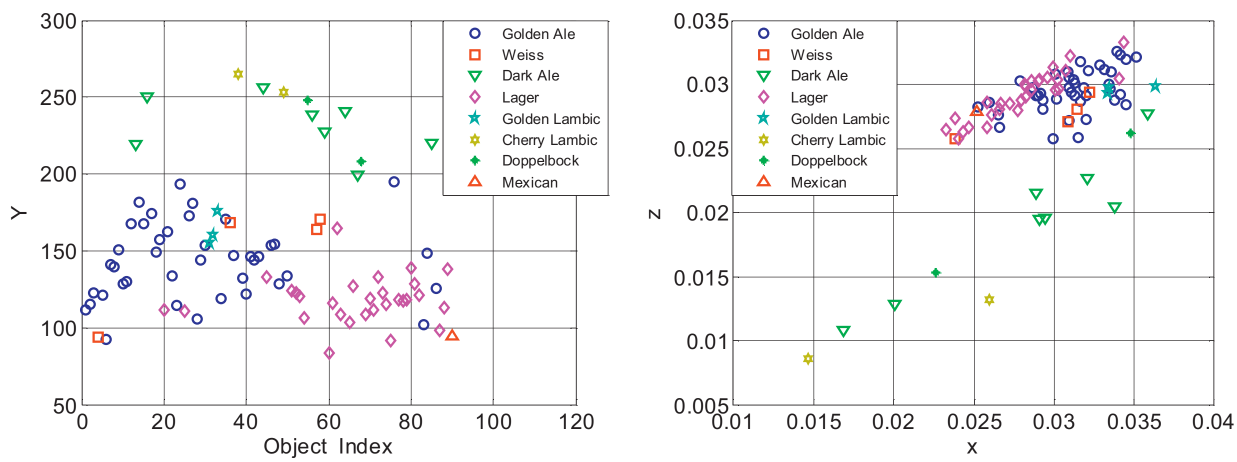

Fig. 4. Results of colorimetric processing: luminance behavior (left) and (*x*–*z*) plot.

a highly undesirable feature, because variables having a skewness of higher than 1 (in absolute value) could weaken the results of the multivariate data processing. Consequently, the turbidity values have been expressed in logarithmic scale in order to achieve both a more uniform spread of the values and a lower skewness.

Refractive index measurements were performed by means of a commercially-available hand-held Abbe refractometer (Atago, model R-5000), equipped with a thermometer for the purposes of temperature compensation. The instrument worked in the 1.33–1.52 refractive index range with an accuracy of ±0.1%. Fig. 6 shows the results of the refractive index measurements, which partly reflect the alcoholic strength. The analcoholic Tourtel shows the lowest refractive index, while the highest alcoholic EKU28 beer (G1) shows the highest refractive index. Fig. 7 shows the

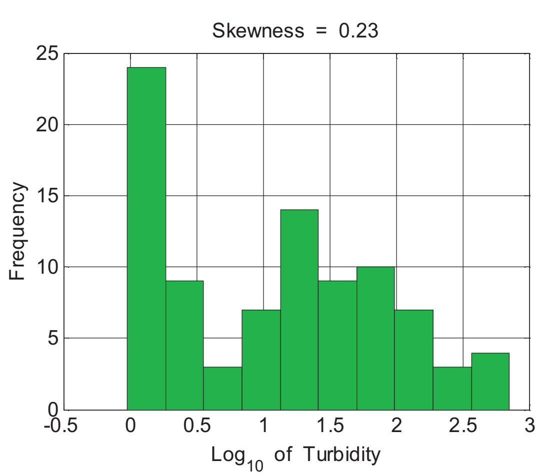

Fig. 5. Logarithm turbidity values of the beer collection.

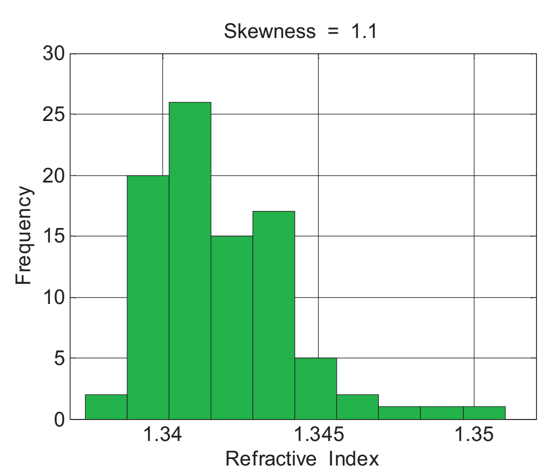

Fig. 6. Refractive index values of the beer collection.

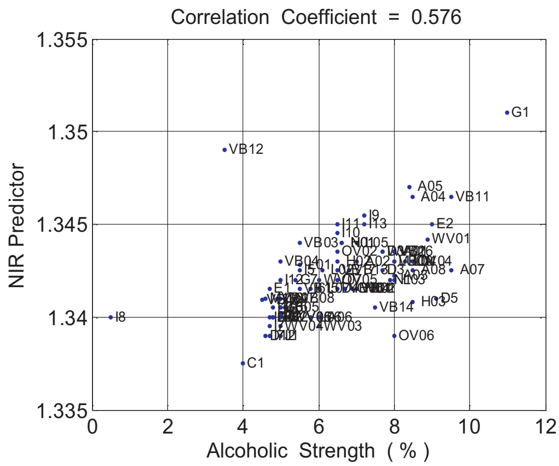

Fig. 7. Relationship between refractive index and alcoholic strength.

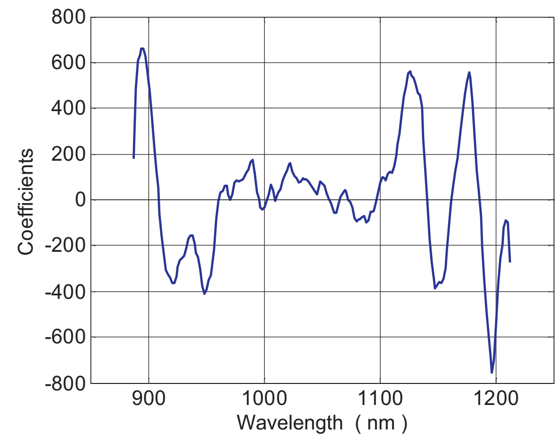

Fig. 8. Regression coefficients used in Eq. (1) as a function of wavelength.

relationship between refractive index and alcoholic strength, exhibiting a correlation coefficient of 0.576. This low value was mainly caused by the sugar content, which also influences the refractive index. In fact, by removing the low-alcoholic fruit-flavored Kriek Lindemans beer (VB12), the correlation coefficient increased up to 0.677.

The measured turbidity and refractive index values are explicitly summarized in Table 1.

3. Results and discussion

3.1. Predicting the alcoholic content

A quality indicator of beer is its alcoholic content. Optical spectroscopy in the near-infrared band, which was conventionally carried out in transmission mode by means of standard quartz cuvettes, demonstrated its effectiveness for predicting the alcoholic strength of a number of alcoholic beverages, thanks to the strong absorption of ethanol in this band [48–50]. However, since beer is a turbid medium, conventional spectroscopy could lead to misleading results because of the scattering effects, which could vary according to the sample type. On the contrary, the innovative technique of diffuse-light absorption spectroscopy which we used made possible a scattering-free prediction of the alcoholic content.

The Partial Least Squares regression (PLS) was applied to the near-infrared spectra of Fig. 3-right. Indeed, PLS is one of the most popular techniques for the prediction of quantitative variables in a multicomponent mixture and is used when the predictor matrix has many collinear variables and the usual Multiple Linear Regression cannot be applied [51]. PLS looks for a limited number of PLS factors that are linear combinations of the original predictors. These new variables are mutually orthogonal (thus, uncorrelated) and have the maximum possible covariance with the target variable, among all possible combinations of the original predictors.

In practice, this model made it possible to predict the alcoholic content of #n beer by measuring the near-infrared diffuse-light absorption spectrum, and by using Eq. (1)

$$\widehat{y_n} = \sum_{m=1}^{M} r_m x_{nm} \tag{1}$$

where M is the number of predicting variables (the wavelengths); r_m the regression coefficient of the m-wavelength, as shown in Fig. 8; x_{nm} is the absorbance value at m-wavelength of #n beer.

Fig. 9 shows the results of the PLS processing of near-infrared spectra for alcoholic content prediction. The PLS model was calibrated using 48-randomly sorted beers, and results in Fig. 9-left were obtained. The number of PLS factors was obtained by means of a 12-subset random cross-validation, the minimum cross-validation error of which was attained with 4 PLS factors, as shown in Fig. 10. The final model was then tested on the 38 remaining beers, and the results in Fig. 9-right were obtained. Table 2 summarizes the results of the PLS processing.

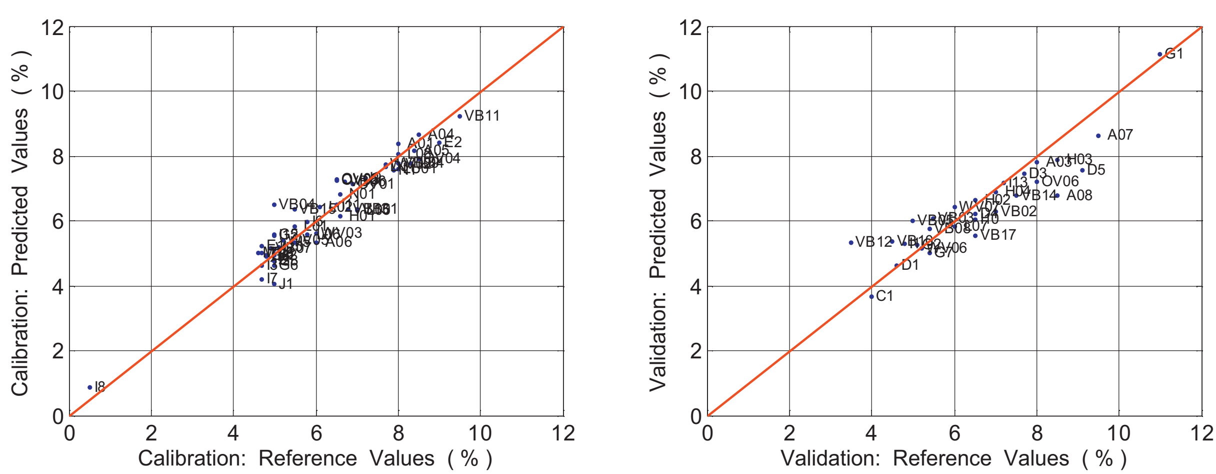

Fig. 9. Prediction of the alcoholic content of beer: results of calibration (left) and validation (right) models.

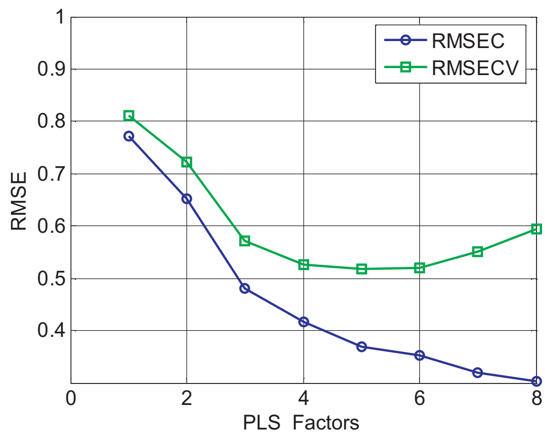

Fig. 10. Root Mean Square Error as a function of PLS factors for both calibration and validation.

Table 2
Prediction of the alcoholic content of beer: summary of PLS regression coefficients and their meaning.

Coefficient	Value	Meaning
R^2	0.93	Squared correlation coefficients between predicted and reference values, for the calibration set. The fit is as better as this value is closer to 1.
RMSEC	0.4	Root Mean Square Error of Calibration, representing the spread of calibration points along the target line. The fit is as better as this value is closer to zero.
RMSEP	0.5	Root Mean Square Error of Validation, representing the spread of validation points along the target line. The fit is as better as this value is closer to zero; also, the calibration/validation model is as better as RMSEC and RMSEP are closer.

3.2. *Identifying the beer character*

An explorative analysis was carried out by fusing the experimental data which were intuitively correlated to the beer identity, and then applying Principal Component Analysis (PCA), which is one of the most popular methods for data dimensionality reduction and straightforward explorative analysis [52]. The chosen variable for beer characterization were: color, alcoholic strength, turbidity, and refractive index. As far as the alcoholic strength is concerned, instead of using all the variables of Eq. (1), only the most significant wavelengths were employed in order to create a "simplified" predictor.

An 86×6 matrix was created, by considering the following 6 variables as particular information of each beer identity:

1. TURB = $\log_{10}$(turbidity).
2. IND = refractive index, which is related to the Brix and to the alcoholic content.
3. NIR = D_1 (894 nm)–0.6 × D_1 (921 nm)–0.6 × D_1 (953 nm) + D_1 (1126 nm) + 0.8 × D_1 (1177 nm)–D_1 (1197 nm) where $D_1(\lambda_i)$ were the first derivatives of the near-infrared spectrum at the wavelengths showing the most significant regression coefficients for alcoholic content prediction (see Fig. 8). The coefficients of the combination are the heights of the regression coefficients at the chosen wavelengths, relative to 894 nm.
4. *Y*.
5. *x*.
6. *z*.

Where *Y*, *x*, and *z* represent the indicators of the beer color.

This data matrix was then processed by means of PCA. Fig. 11 shows the results of processing in the PC1-PC2 subspace. Fig. 11-left shows the score-loading biplot in which the scores and loadings are scaled in order to enable the simultaneous representation of samples and variables in the same plot. The samples are represented as points, while the variables are represented as vectors emanating from origin and indicating the direction in which the corresponding variable increases. Fig. 11-right shows the score plots only: this is a zoom of the previous figure without the variable-vectors, and provides a clearer interpretation of the results.

The PC1-PC2 plane, which explains about 73% of the total variance, distinguishes the Lager, Golden Ale and Dark Ale beers quite well:

- Lager and Golden Ale beers are separated along the bisecant of quadrants II and IV. The main contribution to this splitting comes from NIR, due to the higher alcoholic content of Ales. TURB and IND also have a strong influence.
- The distinction between Dark and Golden Ales is along the bisecant of quadrants I and III, and is mainly due to chromaticity. Red beers have stronger *Y* and weaker *z* and *x* than golden beers. NIR, IND and TURB are similar for both red and golden beers.
- Weiss and Golden Lambic beers cannot be separated from Golden Ales. Indeed, Weiss and Ale beers are similar because they are top-fermented beers. Moreover, Weiss beers are never 100% wheat

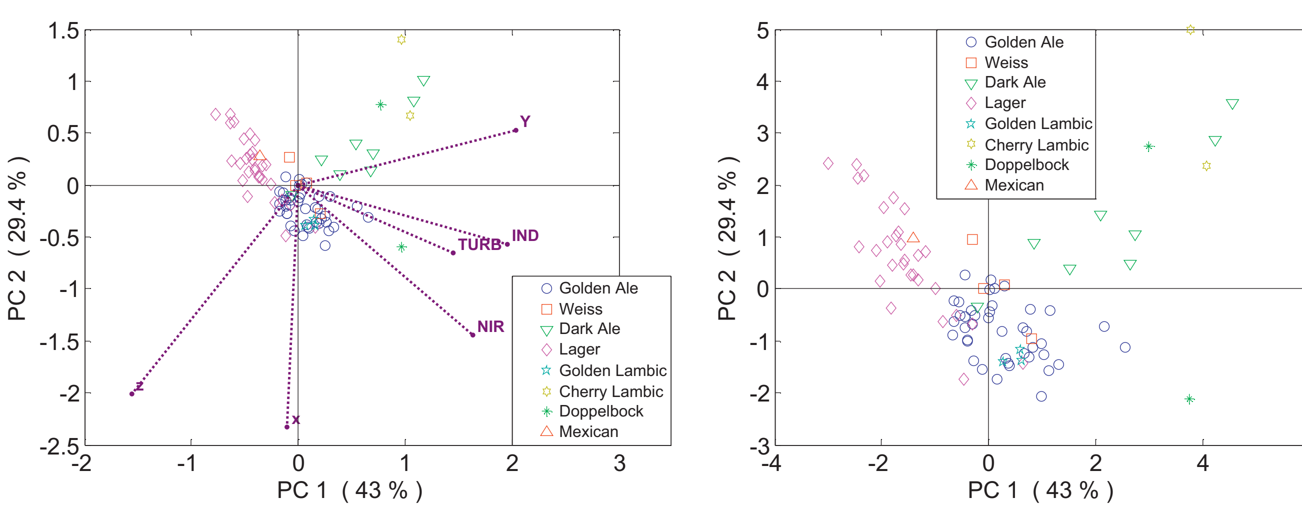

Fig. 11. Beer clustering according to class in the PC1-PC2 plane: score-loading biplots (left) and score plots (right).

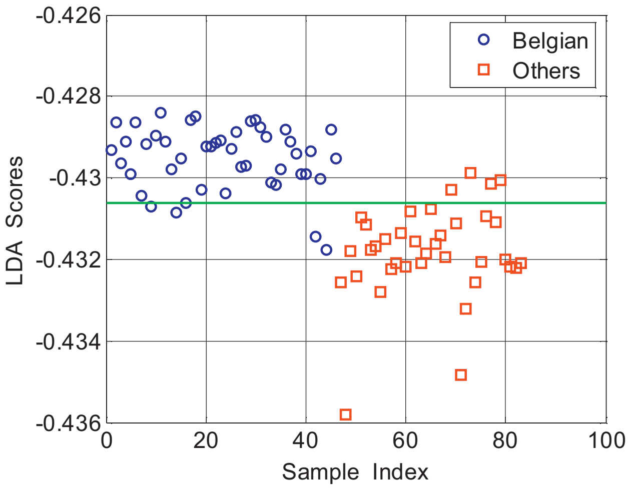

Fig. 12. Distinguishing Belgian beers from all the others.

(the wheat percentage is about 50%), and some Ale beers also contain wheat. They are, in fact, made by means of similar recipes, and the difference between them is a matter of wheat percentage.

- The "Corona" Mexican beer is a "Pale Lager". Therefore, it is correctly classified in the same area as Lager beers.
- Red beers are more scattered than the others, because their chromatic differences are larger.
- A particularly wide spread was found within the Doppelbock and Cherry Lambic groups. This was mainly caused by strong differences in the NIR and IND variables, which were influenced by their different alcoholic strengths and sugar content.

3.3. Distinguishing the Belgian beers

Belgian beers are peculiar in several aspects. In fact unique herbs, spices and fruits are often used in brewing them, as well as pale and dark candy sugars, caramelized or aromatic malts and honey. Also, the use of wooden vessels and the practice of bottle conditioning are typical of the Belgian traditions. Not to mention waters: after all, Belgium is renowned as the land that gave the world "Spa" [13]. All these factors influence the organoleptic properties of beer and, potentially, their optical properties as well.

Distinguishing the Belgian beers was achieved by using the same fused-data matrix previously considered for the identification of beer characteristics. This matrix was processed in this case by means of Linear Discriminant Analysis (LDA), which is a robust and reliable technique for automatic object classification [53]. Seven beers (A04, VB17, WV01, VB12, I9, I11, I10) had to be removed from the data set, because their saturated absorption in the 400–500 nm band altered their tri-stimulus coordinates. Although this saturation had little importance for the previously-presented explorative analyses, it spoiled the performance of the LDA classification run. Consequently, the data set for LDA analysis was reduced to 79 samples.

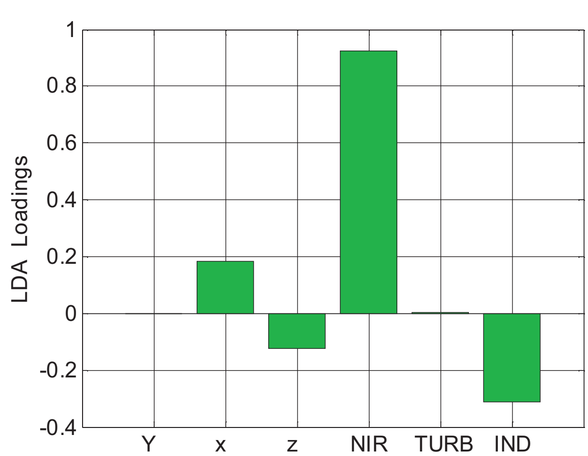

Fig. 13. Loading plot of LDA processing.

Fig. 12 shows the results of the LDA processing: 9 out of 79 beers were misclassified (about 11%), and the cross-validation test provided a misclassification rate of 13%.

Fig. 13 shows the loading plot of the LDA processing. It is interesting to note that the variable Y (luminance) has negligible weight: in fact, both the Belgian and the other beer classes include clear and dark beers. The variable NIR has the predominant weight, because the average alcoholic strength is higher for Belgian beers. Although also turbidity is generally higher for Belgian beers, this variable shows a very high intra-class spread, thus reducing its contribution.

These results demonstrate that optical methods can provide an effective tool for beer discrimination according to the country of production.

4. Conclusions

A miscellaneous assortment of 86 beers was considered. They were produced in different countries using different ingredients, recipes, and fermentation methods. All beers were characterized by means of an innovative setup for diffuse-light absorption spectroscopy, which provided a scattering-free spectroscopic fingerprint of beers in the visible and near-infrared bands. In addition, standard turbidity and refractive index measurements were performed. All these data were processed by means of multivariate data analysis in an attempt to highlight peculiarities and characteristics of beers.

The ability to predict alcoholic strength by means of a straightforward processing of the near-infrared spectra processing was the first encouraging result achieved. This result demonstrates the possibility of scattering-free detection of the alcoholic content during the brewing, which can be performed continuously and online without having to worry about beer turbidity.

The fusion of scattering-free optical data, which also included a color information, together with the turbidity and refractive index values, and a simple processing based on standard PCA and LDA analyses demonstrated the possibility of achieving a good grouping according to beer class, and of distinguishing the beers produced in Belgium from all the others. These preliminary results are promising: they represent a first attempt at correlating optical data to beer quality indicators addressed to authentication actions.

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Anna Grazia Mignani was born in Bologna, Italy, in 1957. She holds a Laurea in Physics and a PhD in Non Destructive Testings from the University of Florence, Italy. Since 1983 she works at CNR-IFAC (formerly IROE), at present as senior scientist. Her research work includes fiber optic and micro optic sensors, passive guided-wave components for sensing applications, and fiber optic sensor networks. This activity is documented by many journal and conference publications, invited talks, and some international patents. She managed national and international research contracts on application oriented optical sensing. She chaired the 14^{th} International Conference on Optical Fiber Sensors, some SPIE Conferences on Optical Sensing, and she served as Guest Editor of IEEE Sensors Journal and IOP Measurement Science and Technology. She is a member of the Board of the Italian Society of Sensors and Microsystems.

Leonardo Ciaccheri received both his degree in Physics and his PhD degree in Non Destructive Testings from the University of Florence, Italy. The former in 1997 and the latter in 2001. From 2003 to 2009, he collaborated continuatively with the Fiber Optic Group at CNR-IFAC, where he became permanent-staff scientist in 2009. His current research interests are in fiber optic sensors for spectroscopy and multivariate data processing. He also collaborates with the faculty of Electrical Engineering of the University of Florence, where he teaches geometric optic, as part of the optoelectronic course.

Andrea Azelio Mencaglia received his degree in physics from the University of Florence in 1987. In the period 1987–1996 he was with CNR, at IFAC (formerly IROE), first awarded with fellowships and then as researcher. In 1996, he was Research Fellow at the University of Strathclyde, Glasgow, UK, for 6 months, within the Human Capital Mobility program. From January 1997 to 2001, he was responsible for the research activity at Prodotec, a small Florentine enterprise. Since January 2002 he is permanent-staff scientist at CNR-IFAC. His activity is in the area of optical fibers sensors and systems and optoelectronic instrumentation (applications in the fields of diagnostics for food, cultural heritage, environment, industry and medicine. He has been scientific responsible in several research contracts with companies operating in the clinical diagnostics and collaborates in several European and National projects. He is author of more than 100 publications in Journals and Conference Proceedings and of several patents (US, Europe and Italy).

Heidi Ottevaere obtained in 2003 the PhD degree in applied sciences from the Vrije Universiteit Brussel (VUB), Brussels, Belgium for her work entitled: "Refractive microlenses and micro-optical structures for multi-parameter sensing: a touch of micro-photonics". Afterwards she continued her career as a post-doc researcher and from 2009 onwards as full professor at VUB. She made research efforts in a large variety of fundamental and applied research topics, most of them situated in the domain of micro-photonics and micro-optics. In particular she has built up expertise in the following research topics: plastic micro-optics based on Deep Proton Writing, interferometric measurement techniques, microlens characterization and biophotonics.

Edgar Eugenio Sámano Baca graduated in 2009 from the Bachelor's program in Mechatronics Engineering in the Universidad de las Américas Puebla (UDLAP), Mexico for his work entitled: "Design and Simulation of an Accelerometer Using MEMS Technology". Currently, he is pursuing his academic career as a MSc Photonics Engineering student at the Vrije Universiteit Brussel (VUB), Belgium. During the past 2 years, he has studied various fundamental and applied photonic subjects and performed mainly research in the area of spectroscopy.

Hugo Thienpont is a full professor at the Faculty of Engineering of the Vrije Universiteit Brussel (VUB), Brussels, Belgium. He chairs the Applied Physics and Photonics Department and is director of its photonics research group. He is internationally recognized for his breakthroughs in the following research topics: optical bistability in nonlinear Fabry-Perot resonators, nonlinear optical response of organic materials, parallel optics in digital computing demonstrators, optical interconnects at the chip level, high-aspect ratio plastic micro-optics based on deep proton lithography, polarization switching in vertical cavity surface emitting microlasers, and micro-structured optical fibers for optical sensing.