PHOTONICS-ENHANCED SENSORS FOR MONITORING FOOD QUALITY AND SAFETY

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Abstract – Food quality and food safety are gaining the past decades more and more importance. In particular application domains such as the identification of foreign bodies in solid food streams, the quality screening of vegetables and fruits, the recognition of food products inducing a health risk and the monitoring of the quality and authentication of liquids. In view of this, the authors of this paper started exploring the potentialities of photonics where the main objective consisted in answering the question if food screening methods could be photonics-based.

Keywords: photonics, optical sensors, food, quality screening, food contamination

1. INTRODUCTION

The safety of both solid and liquid food products is crucial, therefore a continuous monitoring is needed. Indeed, one could look to the identification of foreign bodies in food streams, the quality screening of solid food products, the identification of food products inducing a health risk and the quality monitoring and authentication of liquids. In this paper we demonstrate with the help of three concrete case-studies the usefulness of optical screening methods to perform the quality screening of solid food products as well as the identification of food contaminants inducing a health risk.

2. QUALITY SCREENING OF FOOD PRODUCTS: THE USE OF VEGETATION INDICES TO ESTIMATE THE MATURITY OF GREEN VEGETABLES

2.1. Introduction

The quality of green vegetables is strongly related to ripening and senescence resulting in a change in pigment composition and pigment concentration. During these processes different bio-chemical reactions take place with the most important ones the conversion of chlorophyll a molecules into chlorophyll b molecules and in a later phase the breaking-off of the chlorophyll molecules and the accumulation of carotenoid molecules. This implies that if we aim an optical based maturity classification of green vegetables, we need to monitor differences in pigment type and concentration. A comparison can be drawn with the monitoring of stress, senescence and diseases in higher plants. Extensive research has been conducted to estimate in a non-destructive way the chlorophyll and carotenoid content of vegetation. These studies have resulted in the definition of so-called 'spectral vegetation indices' that indicate the pigment concentrations.

2.2. Materials and Methods

The quality screening of food products will be discussed in detail using one concrete example. The test group of sample under test consists of Berlotti beans in four different states of maturity (fresh=dark green color, less fresh=green color, overripe=light green color, strongly overripe=yellow color).

The objective of this research is to evaluate the class differences (quantitative values to describe the performance of a certain optical measure) between these four subgroups applying different types of optical measures. Particularly, we study the following indices:
- The color indices r, g and b.
- The Normalized Difference Vegetation Index (NDVI) is calculated from the ratio between the difference and the sum of the reflectance at two different wavelengths; respectively coincident and non-coincident with the chlorophyll absorption band. The red reflectance value is negative correlated with the chlorophyll concentration while the infrared reflectance value is positive correlated with the internal scattering of the product. For reasons of commercial availability we selected 660 nm and 780 nm as detection wavelengths.
- The 'Red Edge Inflection Point Position' (REP) equals the wavelength that defines the transition between the region of high absorption (situated in the visible part of the spectrum) and the region of high reflection (near-infrared region). In practice one

takes the inflection point as a quantitative value; this value corresponds to the wavelength for which the second derivative of the reflectance spectrum becomes zero. The REP is typically situated between 690 and 740 nm. For each subclass we measure the reflectance spectra between 400 nm and 800 nm for 30 samples making use of the set-up described in [1].

2.3. Discussion of measurement results

An overview of the different calculated indices is given in Table 1.

Table 1. Calculated mean and standard deviation values of the color and vegetation indices of Berlotti beans in four stages of ripeness.

parameter	fresh	less fresh	overripe	strongly overripe
r	0.353 ± 0.013	0.427 ± 0.036	0.490 ± 0.038	0.586 ± 0.013
g	0.502 ± 0.022	0.412 ± 0.034	0.351 ± 0.034	0.2568 ± 0.0095
b	0.1448 ± 0.010	0.1611 ± 0.0065	0.1590 ± 0.0077	0.1569 ± 0.0086
NDVI	0.675 ± 0.026	0.471 ± 0.067	0.267 ± 0.076	0.0846 ± 0.0098
REP (nm)	690.53 ± 0.7	688.88 ± 0.66	686.57 ± 0.78	673.53 ± 0.20

The color density parameters r, g and b agree with our physical observation of the products: the greener the product, the higher the g parameter and the lower the r parameter. The b parameter has a constant value for all classes. It is clear that these values do not allow the separation of the four subgroups; both g and r values have overlapping values for beans belonging to the 'overripe' and 'less fresh' product group. However, applying the two vegetation indices NDVI and REP enable the differentiation between all groups. We expect that the absorption peak in the red spectral region becomes broader and less steep as the bean gets riper and that consequently the REP and the NDVI values will decrease.

Table 2. The vegetation indices NDVI and REP result in the highest class difference D.

D	*R*	*g*	*b*	*NDVI*	*REP* (nm)
fresh-less fresh	4.32	4.97	3.02	6.35	3.83
fresh-overripe	10.26	10.36	2.75	15.28	9.94
fresh-strongly overripe	32.72	23.84	2.27	49.06	53.23
less fresh-overripe	3.14	3.28	0.55	5.31	6.01
less fresh-strongly overripe	9.57	10.01	1.06	12.83	50.85
overripe-strongly overripe	7.56	8.44	0.58	7.53	51.21

The measurement results consolidate these expectations. Comparison of the class distances D for the different parameters (Table 2) consolidates the suitability of both vegetation indices. The NDVI and the REP values result in the highest class differences.

2.4. Implementation in scanning engine

Implementation of the NDVI in a scanning engine for screening the quality of green vegetables is only requiring the detection of two light intensities at the two mentioned wavelengths. Realizing the measurement of the REP is a more challenging task since we need to measure a spectrum consisting of multiple points. We designed a system of which the concept consists of using a commercial available multi-anode photomultiplier tube (32 element MA-PMT - Hamamatsu) in combination with a dispersive grating (1200 lines/mm) to sense multiple wavelengths at once. An additional lens (achromatic with f=76.2mm) is needed for collimating and focusing purposes. The 'normalized difference vegetation index' (NDVI) and the 'red edge inflection point position' (REP) are two vegetation indices that allow the classification of green vegetables owing different maturity levels.

3. IDENTIFYING FOOD CONTAMINANTS INDUCING A HEALTH RISK: THE USE OF FLUORESCENCE TO DETECT AFLATOXINS IN FOOD PRODUCTS SHOWING LOW NATURAL FLUORESCENCE

3.1. Introduction

Analysis for mycotoxins is essential to minimize the consumption of contaminated food and feed. As most mycotoxins are toxic in very low concentrations, sensitive and reliable methods are required for their detection. The European Union (EU) has established with the Commission Regulation No. 1881/2006 severe limits for major mycotoxin classes in many products at high risk of contamination. Also the US Food and Drug Administration (FDA) has specified safety regulations.

Mycotoxin analysis is mostly performed by in-laboratory diagnostic analytical tools based on thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). Despite their high sensitivity, the disadvantage of these techniques is that they are invasive (extensive sample treatment is required), expensive, slow and labor intensive, such that they can only be applied to a limited amount of

samples. Moreover it is known that the toxins tend to develop in a very uneven distribution which makes that it is not certain at all that a sample taken for analysis is truly representative of the whole consignment. Because of this several efforts were made in recent years to investigate the possible use of optical detection methods which allows a faster identification of the toxins and as such pave the way for an individual screening of a larger amount of samples. In general two different techniques appears to be valuable: fluorescence and near-infrared spectroscopy.

After the successful implementation of chlorophyll fluorescence to separate foreign objects from green vegetables, the question arose if aflatoxin fluorescence, and more in particular one-photon induced fluorescence (OPIF), could be used to detect toxic food products. Aflatoxins belong among ochratoxin A and citrinin to the group of fluorescing mycotoxins [2]. Aflatoxins consist of a group of approximately 20 related fungal metabolites of which only aflatoxins B1, B2, G1 and G2 are normally found in foods including cereals, maize, nuts (groundnuts and pistachios), spices, figs and dried fruit. They are named after their fluorescent colors (B for blue and G for green).

3.2. Materials and methods

The target is to sense the presence of aflatoxin B1 in groundnuts since this mycotoxin is the most toxic one. The absorbance spectra of aflatoxins dissolved in ethanol are published [3]. Fluoresce light is emitted in the spectral region between 400 and 500 nm after excitation in the ultraviolet range between 200 and 400 nm.

To excite the aflatoxin molecules we illuminate the test samples with an argon ion laser system (model 2030 - Spectra Physics). After mounting the matching UV mirror set, this laser emits two wavelengths: 351.1 nm and 363.8 nm. Because both wavelengths are very close to the aflatoxin's absorption maximum, we expect high emission levels. In Fig. 1 these two emission lines are displayed together with the expected absorbance and emission values for aflatoxin B1 in ethanol (values are taken from [3]).

For each of the two subgroups of available test material (non-contaminated and contaminated groundnuts) the fluorescence spectra of 30 samples are measured using the set-up described in [1].

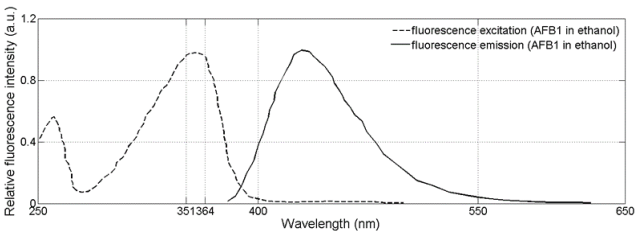

Fig. 1. The 351.1 nm and 363.8 nm emission lines of the argon ion laser with UV mirror set are closely positioned to the absorption peak maximum of aflatoxin B1. Aflatoxin B1 fluorescence is emitted between 400 and 550 nm [3].

3.3. Discussion of measurement results

Analysis of the averaged fluorescence spectra leads to the following conclusion. Although both groups emit a blue-green fluorescence signal (400-550 nm), a difference in spectral shape is observed. An intersection point is present close to 430 nm (Fig. 2). In the spectral region before this point (410-430 nm) the contaminated nuts fluoresce most; the contrary is true in the subsequent spectral emission region (430-520 nm). This is a first indication that the ratio between the fluorescence intensities in the described spectral regions can act as a potential candidate to detect groundnuts contaminated with aflatoxin B1.

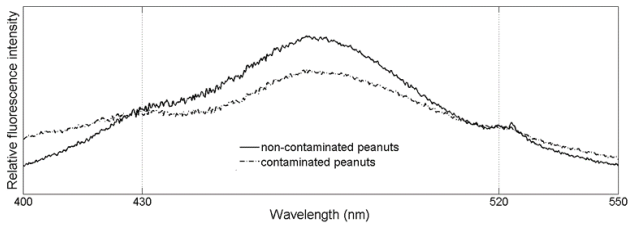

Fig. 2. A spectral shape difference in fluorescence spectrum is observed between the contaminated and healthy groundnuts. Close to 430 nm both curves intersect.

3.4. Implementation in scanning engine

The spectroscopic outcome described above was fine-tuned by our industrial partner who prototyped and commercialized this technology in a scanning-based optical detection engine. A fast optical screening method was established that allows the identification of groundnuts contaminated with aflatoxin B1.

3.5. Towards two-photon induced fluorescence

Recent research has shown that two-photon induced fluorescence (TPIF) represents a promising technique for the detection of mycotoxins in complex samples. This technique implies the simultaneous absorption of two photons of identical or different frequencies in order to excite the fluorescence molecules. In contrast to the regular OPIF, the background luminescence signal of the matrix may be successfully eliminated or at least reduced. Besides the reduction of the background emission, a second advantage of using two-photon induced fluorescence is the shift of the UV excitation wavelength towards longer values which can more easily be found on the commercial market.

Maize is a product type that displays high levels of natural fluorescence. We have considered two maize batches with a different origin and harvested during two consecutive years. Each batch contains a healthy and a contaminated (aflatoxin B1) subsample. Prior to the optical analyses the aflatoxin levels of each subsample are verified using a commercial available laboratory test system (ToxiQuant - ToxiMet). After the optical study, the contamination of the samples was confirmed by the CODA-CERVA, the Belgian Reference Laboratory for Mycotoxins. The details of the research outcome are published in two peer-reviewed scientific papers [4, 5]. Hereafter, we give a resume of the most important findings.

- Two-photon absorption can be induced in maize contaminated with aflatoxin B1. This is illustrated in [5] where the integrated fluorescence intensity (400-600 nm) is plotted as a function of the excitation power, maintaining a constant spot size close to 1 mm for the OPIF and close to 0.2 mm for the TPIF measurements. The studied excitation wavelengths are 365 nm (OPIF) and 730 nm (TPIF). The integrated OPIF intensity linearly increases with the excitation power until a value close to 35 mW where saturation sets up. The integrated TPIF intensity displays a quadratic dependence on the excitation power confirming the occurrence of the non-linear two-photon absorption process [5].

- TPIF requires a higher illumination power density compared to the OPIF conditions (~ factor 100-200). In addition the emitted fluorescence signals are approximately 100 times lower [5].

- Both the healthy and the contaminated samples show fluorescence emission. Though, the spectral shapes of both product groups are different since the aflatoxin molecules change the fluorescence properties of the matrix elements [4], [5].

- Separation between the healthy and contaminated products is possible by selecting the ratio of the emitted fluoresce signals in the spectral regions 400-475 nm and 475-550 nm as optical sensing parameter [4]. TPIF gives a slightly larger intensity contrast compared to OPIF [5].

4. MONITORING QUALITY AND AUTHENTICATION OF LIQUIDS

4.1. Introduction

Next to solid food products the monitoring of liquids in the food cycle is of crucial importance. For example vinegar is an important condiment in modern and traditional cuisines of the world and can be fermented from various foodstuffs such as wine, cider, fruit musts, malted barley, rice or pure alcohol according to the region and local customs. The pigments of vinegar are mainly caused by the following factors: the color of the original materials, the pretreatment process, the fermentation process, and the addition of caramel pigments. For vinegars, the application of optical spectroscopy is mostly focused on counterfeiting detection and determining the type and origins of unknown samples. In this paper, we will demonstrate that by downscaling bulky laboratory analyses on an optofluidic chip in combination with multi-measurement analysis, it is possible to identify vinegars.

4.2. Materials and methods

For these experiments we have chosen a set of 9 vinegars of which the sample plug is pushed into the system described in [6] by ultra pure water (Merck LichroSolv). For each measurement, the same injection protocol is respected, injecting a sample plug of 70 mL at 15 mL/s. All vinegar samples are commercially available (Oil & Vinegar brand, Belgium).

After measuring the signal responses observed by each detector for the different types of vinegar in the

time domain, we calculated the FFT of each detector's signal envelope. This transformation to the frequency domain is done to counter the effects that viscous fingering has on the signal envelope. The plot of this dataset indicates where the data from different detectors is located and where the DC values can be found.

In a next step we have applied multivariate data analysis. Principal Component Analysis (PCA) will automatically apply weights to the frequencies that are significant [6]. In figure 3 we show the PC plot of the experimental data obtained from the collection of vinegars. When we look at the PC plot of the vinegars we observe clear clustering, making classification of vinegar samples possible.

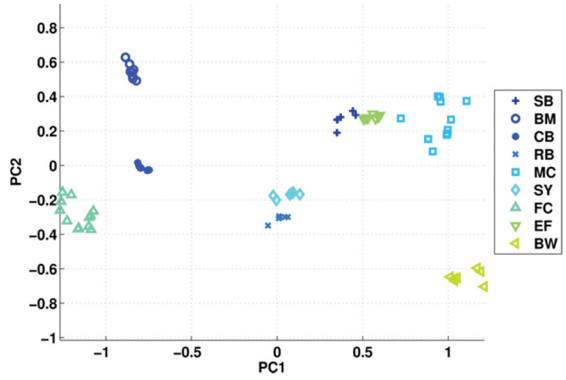

Fig. 3: Principal Component plot of the experimental data of vinegars (SB Strawberry, BM Balsamico di Modena, CB Cranberry, RB Raspberry, MC Marc de champagne, SY Sherry, FC Fig cream, EF Elderflower apple lime, BW White balsamico)

We have shown a detection system that is capable of simultaneous ABS, LIF and scattering measurements excited by two time-multiplexed laser sources at 405 nm and 450 nm for the identification of vinegars. With the help of Partial Least Squares (PLS) it is possible to predict out of this measurement data the viscosity and turbidity of the vinegar samples. The viscosity of vinegar is an important quality parameter and also an indicator of the progress of its production process. A vinegar's turbidity value can provide information on how well it is filtered during production, furthermore besides a vinegar's color, turbidity will significantly impact the final product's appearance

5. CONCLUSIONS

This paper shows that photonics-based food sensors can definitely contribute to the enhancement and safety of both solid and liquid food products. During the conference more concrete examples will be highlighted.

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