FOOD ANALYTICAL METHODS (ISSN: 1936-9751) 8(9): 2243-2249 (2015).

DOI: 10.1007/s12161-015-0102-1

pH and CO_2 sensing by curcumin coloured cellophane test strip

Mária Pávai, Judith Mihály and András Paszternák*

Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, 1117 Budapest, Magyar tudósok körútja 2., HUNGARY

**Corresponding author: paszternak.andras@ttk.mta.hu, Tel./Fax: +36-1-382-6988*

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ABSTRACT

Curcumin coloured cellophane test strip - fabricated without any synthetic material - is used to determine *in situ* the instantaneous pH changes in liquids and in the air. A low cost, portable sensor array for possible medical and food-industrial application is developed. Next to the pH detection, curcumin coloured test strip can be applied as CO_2 monitoring sensor, too. On-going processes and the structural changes are followed by UV-VIS spectrophotometer, ATR-FTIR and AFM.

KEYWORDS: pH sensor, curcumin, cellophane, CO_2, medical application, food quality

1. Introduction

Based on previous experiments, cellophane shows selective colouring effect to synthetic and natural colours: test strip is coloured in case when molecule of the dye contain aromatic azo-groups (e. g. tartrazine, azorubine) or aromatic groups (e. g. patent blue V, *curcumin*) with resonance effect inside the molecule; test strip is left uncoloured by dye without aromatic azo-groups (e. g. β-carotene), aromatic groups (e. g. capsorubin, caramel) and those which contain aromatic groups but without resonance effect inside molecule (e. g. betanin, riboflavin). Taking advantage of this property, in this paper the pH sensitivity of the curcumin coloured cellophane is discussed.

1.1 Advantages of curcumin

Curcumin is one of the strongest active ingredient in the curcuma root. Curcumin has intensively been investigated, thanks to its impact on body health (Soni et al. 2011): for its anti-oxidant properties; because it appears to slow the growth of tumour cells (Naksuriyaa et al. 2014; Salem et al. 2014) and even to be able to kill them; it can lower cholesterol levels and reduce obesity; able to stimulate muscle regeneration after trauma (Thaloor et al. 1999) corrugate skin diseases; is a healing herb for the liver, spleen, stomach, intestines, lungs and blood; can prevent and cure Alzheimer's disease and can reduce inflammatory reactions (Ammon et al. 1993).

Intensive research is also done on packaged sticker sensors based on natural dye of curcumin (Kuswandi et al. 2012). In this case curcumin is used as a chemical sensor for the detection of volatile inorganic and organic amines produced during bacterial growth in shrimp samples.

1.2 Most common methods for pH testing

pH is a unit of measure which describes the degree of acidity or alkalinity of a solution. A rough indication of pH can be obtained using pH papers or indicators, which change colour as the pH level varies. More accurate measurements can be performed by pH meters working on electrochemical basis (Sigel et al. 1991). The first pH meter is constructed in 1934 by A. Beckman (ACS 2004). The glass pH electrode, having H^+ ion activity dependent potential, is constructed much earlier, in 1906 by F. Haber and Z. Klemensiewicz (Stock 1991).

Qualitative pH determination still find different applications also in the era when pH tester at nanometric level are developed (Bishnoi et al. 2006). It is suspected that the pH paper is first invented in the early 1800s by a French chemist named J.L. Gay-Lussac (Szabadváry and

Oesper1964; Crosland 1978). Two types of pH paper are commonly used: litmus paper and universal (alkacid) paper (Foster and Gruntfest 1937).

Visual pH sensing methods using natural dyes are in the focus of ongoing research (Zhang et al. 2014) thanks to their convenient and non-destructive character.

1.3 Characteristics and the use of cellophane

Cellulose $(C_6H_{10}O_5)_n$ is a colourless, odourless and non-toxic natural polymeric solid (Je and Kim 2004) found in plant cell walls. The cellophane, which is a thin, transparent, regenerated cellulose film produced from sodium cellulose xanthate (Laity et al. 2000), has been an important industrial material for many years. It is used as packaging material for food and confectionary products; due to its good mechanical properties and hydrophilicity it finds applications in batteries industry (membranes) (Beach et al. 2000; Tomé et al. 2011), and in medicine (semipermeable membranes for haemodialysis) (Tomé et al. 2011; Cañas et al. 2002).

Compared to cellulose, it has a differentiated 'skin-core' layer structure, with relatively thin, dense skin on both side of the thicker, porous core. The swelling of cellophane in water is well known, reaching equilibrium within a period of 2 hours, with no further changes in dimensions (Laity et al. 2000).

Cellophane is transparent both to ordinary and UV-light; it exhibits the anisotropic properties of cellulose due to its two optical axes and it has excellent dielectric properties. *Per se* it is not porous, but it contains numerous capillaries, which during the swelling are filled with the liquid (Evans 1964).

The goal of the present study is to develop a quick, qualitative pH sensor using the indicator properties of curcumin (Pourreza and Golmohammadi 2015) coloured cellophane test strip.

2. Materials and methods

2.1 Preparation of the curcumin test strip

Cellophane (Sigma-Aldrich, Budapest, Hungary) is cut to 1x2 cm^2 pieces and is used without further cleaning or surface modification. The manipulation of the test strip is performed with tweezers to avoid the contamination of the sample surface. The strip is immersed in aqueous solution (5 g curcuma powder in 250 cm^3 Milli-Q water (18.2 MΩ·cm)) of curcuma (Kotányi Hungária Ltd. Budapest, Hungary; coloured by E100 – curcumin: *(1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione*) for 24 hours, washed in Milli-Q water and dried at room temperature using blotting paper.

2.2 Application of the sensor

2.2.1 Sodium bicarbonate

5 g of $NaHCO_3$ (Sigma-Aldrich, Budapest, Hungary) is dissolved in 50 ml Milli-Q water.

2.2.2 Test strip stability

The cellophane test strip is immersed into acetic acid (96%, Sigma-Aldrich, Budapest, Hungary) for 24 hours.

2.2.3 Saliva model test

Egg white is separated from the yolk and diluted and stirred with Milli-Q water. To perform the pH changing test 10 g of sugar is added to the egg white.

2.2.4 CO_2 sensing

The curcumin coloured cellophane test strip is immersed into the $NaHCO_3$ solution for 1 minute and later is used directly in a CO_2 rich air environment.

2.2.5 Meat quality test

Fresh chicken breast (SPAR, Bicske, Hungary) is used for the experiment. During rot process, the meat is stored outside of refrigerator for 72 h.

2.3 Visual and UV-VIS colour determination

Fifty parallel measurements of each sample are performed. The test strip samples are washed with Milli-Q water and dried at room temperature using blotting paper. The characterisation is done by an UV-VIS spectrophotometer with 300-800 nm (HP 8452A, Hewlett Packard Palo-Alto, California), the stretched test strip is placed perpendicular to the light path.

2.4 AFM characterisation

The morphology of the cellophane surface is studied with an atomic force microscope (AFM, Pico, Molecular Imaging) in contact mode in air at room temperature using a commercially available cantilever with force constant of 0.12 N/m and a Si_3N_4 tip (Bruker, USA). Images are processed by WSxM software (Horcas et al. 2007).

2.5 ATR-FTIR characterisation

Chemical changes in cellophane structure after curcuma impregnation is followed by infrared spectroscopy. Fourier transform infrared (FTIR) spectra are recorded in ATR (attenuated total reflection) mode by means of a Varian 2000 (Scimitar Series) FTIR spectrometer (Varian Ltd, USA) equipped with a 'Golden Gate' (Specac, Inc, UK) single reflection diamond ATR unit. For spectrum acquisition 4 cm^{-1} spectral resolution and co-addition of 128 individual spectra is used. Before evaluation, all spectra are ATR corrected using the Varian Resolutions Pro 4.0 software package.

2.6 “Wash-out” test

The coloured test strips are immersed into Milli-Q water or in acidic acid for 24 h and dried at room temperature using blotting paper.

2.7 Validation process

Validation measurements are performed using standard pH solutions (Radelkis Kft., Budapest, Hungary: pH 2.10, 5.14, 7.12, 9.35, 10.00 and 11.46 (±0.03 at 25 °C)). Curcumin coloured cellophane test strips are immersed into these standard pH solutions for 30 s, washed in Milli-Q water and dried at room temperature using blotting paper. Each measurement is repeated ten times. The image of cellophane strip is taken by a digital camera (Canon PowerShot A2500) and the picture is analysed using IrfanView software.

3. Results and discussion

3.1 The curcumin coloured test strip

Colouring effect of natural and synthetic dyes on cellophane test strip is studied previously; interesting results are obtained by the application of curcumin as a natural colouring material, so the focus of the present research is shifted to this direction (Fig. 1).

Figure 1.

The morphological differences between the coloured (A1, A2) and non-coloured (B1, B2) test strips are characterised by AFM. The surface of the skin-core structure is slightly modified thanks to the swelling process and the adsorption of the natural dye. The surface roughness (RMS) is changed from 2.9 nm (non-coloured) to 1.9 nm (curcumin coloured) due to clogging of the capillaries by curcumin.

No drastic changes can be observed in the IR spectrum of curcuma impregnated test strip, compared to the one of untreated cellophane (Fig. 2 (a) and (b)).

Figure 2.

Both spectra present characteristic features of cellophane with the broad, overlapped band of –OH stretchings of pyranose rings (3600-3000 cm^{-1}) and with the dominant C-O-C stretching bands at 1021 and 994 cm^{-1}. Spectral subtraction, however, reveals the presence of curcumin (active ingredient of curcuma) by small spectral features (Fig. 3). Here we have to point out that in our experiment natural grinded curcuma has been used without any purification, containing a relative high amount of starch (~67%, USDA 2004) indicated also by the medium strong, broad IR band around 1011 cm^{-1} belonging to C-O stretching vibrations of ester groups (Fig. 3 (b)). Beside the presence of the curcumin, the subtracted spectrum pretends changes in the cellophane structure, too. The well defined –OH stretching bands at 3485 and 3443 cm^{-1} suggests the cleavage of intra- and intermolecular H-bonds. The new bands at 1054 and 987 cm^{-1} in the subtracted spectrum can be assigned to new C-O-C bonds, probably formed with the terminal –OH groups of curcumin molecules. The well resolved band at 888 cm^{-1} can be related to β-glycosidic linkage of cellophane and increases in intensity by increasing structural disorder (Proniewicz et al. 2011).

Figure 3.

The curcumin coloured test strip shows stability in aqueous environment (based on the "wash-out" tests) thanks to the strong interaction between the dye molecules and cellophane. Test strips can be used for a long period of time (months) without the loss of their pH sensing property.

Figure 4.

In acidic environment (Fig. 4) the yellow colour is visibly changed; the uncoloured acidic solution becomes yellow. Until the curcumin is not "washed-out" fully in acidic environment from the test strip, the indicator properties are not drastically affected.

3.2 pH sensing of curcumin coloured cellophane test strip

It is observed that cellophane test strip coloured with curcumin has colour-changing property in different environments. This is in accordance with the well know indicator behaviour of curcumin molecules. In alkaline solution or environment the colour of curcumin turns to red (Fig. 5). Based on the results, colour-changing effect is reproducible, reversible and the process takes place practically immediately.

Figure 5.

As it is presented in Fig. 5 the yellow test strip became orange (Fig. 5B) after the contact with tap water and changed to dark red in $NaHCO_3$ solution (Fig. 5C). Next to visual observation, the colour changes are followed by VIS spectrophotometer. Fig. 5 shows the colouring (a-c) and the indicator effect (d-e) occurring in the core structure of the cellophane strip.

3.2.1 Quick and qualitative determination of saliva pH

In certain situation (after eating; in the case of some illnesses) a qualitative determination of the mouth pH can be helpful. Natural curcumin and cellophane are ideal to perform these quick tests without any health risks.

Due to the fact that our laboratory is not classified for human experiments, egg white is used as model for human saliva. Lysozyme is a component both of human saliva and egg white, too. Yellow test strips colour immersed into the diluted solution of egg white changed immediately to red (change from Fig. 6A to 6B). Due to the prolonged swelling of the cellophane in aqueous environment (discussed in chapter 1.3) time shifted behaviour of the indicator effect is observed, the strip immersed for 24 h has a darker red colour compared to the 30 s sample (Fig. 6B´ compared to 6B).

Figure 6.

To simulate the mouth pH changes after consumption of sweet food products, sugar is added to the egg white (Fig. 6C, 6C'). The pH change is investigated by the cellophane test strip: thanks to the sugar, shift to acidic direction is observed (yellowish colour). Although the colour differences are detectable after 30 s (Fig. 6C), in case of a longer immersion times, these differences between the two stages (egg white, egg white + sugar) are more significant (Fig.6C').

3.2.2 CO_2 sensing

There are several specific work-environments (as food industry, winery), where CO_2 is present in high concentration. A low cost, portable sensor for CO_2 can discover easily the manufacturing process errors or even it can save the life of the employees.

The curcumin test strip is activated in alkaline solution (red colour) and moved to a high CO_2 concentration area (up to a beaker with acidic acid and $NaHCO_3$). The red colour (Fig. 7A) of test strip changed immediately to yellow thanks to the formed CO_2 gas (Fig. 7B).

Figure 7.

When source of the CO_2 gas is removed, the test strip colour changes back to red. To demonstrate the reproducibility, the cycle of the detection is repeated 150 times, resulting the same colour changing.

3.2.3 Quality monitoring of raw meat

Food quality monitoring can be another application of the curcumin coloured test strip. Fresh chicken breast-meat is stored outside of refrigerator for 72 h. To monitor the changes in meat quality, test strip is directly placed first on the fresh, and later the on the rotten slice of meat. In food production, the test strip can be placed directly inside the package of meat products, so the customers can get informed about the freshness without purchase and opening of the

pre-packaged item. On Fig. 8 the differences in the absorbance spectra can be observed due to the different pH of meat: yellow for fresh meat (neutral or slightly acidic) and orange-reddish for rotten meat (alkaline).

Figure 8.

Thanks to the small quantity of the needed materials (2 cm^2 of cellophane and some mg of curcumin) curcumin test strip can be a useful tool in food industry, catering and household.

3.3 Expanding the application of the qualitative method to quantitative method

Colour intensity of test strip is assigned to pH values of standard pH solutions. As shown in Fig. 9 the calibration graph is linear in the pH range higher as 7, similar as it is observed in the case of lab-on-paper coloured by curcumin nanoparticles (Pourreza and Golmohammadi 2015).

Figure 9.

The relative standard deviation for ten replicate measurements with different standard pH solutions is less than 2.8 %.

Conclusions

A qualitative, quick, pH sensing procedure with high sensitivity is presented based on the indicator effect of the curcumin coloured cellophane strip. The coloured test strip presents a durable stability in neutral and alkaline environment and can be used several times thanks to the reproducibility and reversibility of the on-going processes.

The developed method is applied for saliva model system (medical application); for CO_2 sensing (industrial application), and as a meat quality tester (food quality control).

Compliance with Ethics Requirements

Mária Pávai declares that she has no conflict of interest. Judith Mihály declares that she has no conflict of interest. András Paszternák declares that he has no conflict of interest.

This article does not contain any studies with human or animal subjects.

References

American Chemical Society /ACS/ (2004) National Historic Chemical Landmarks. Development of the Beckman pH Meter, American Chemical Society, Washington https://www.acs.org/content/dam/acsorg/education/whatischemistry/landmarks/beckman/beckman-ph-meter-commemorative-booklet.pdf

Ammon HP, Safayhi H, Mack T, Sabieraj J (1993) J Ethnopharmacol 38:113-119

Beach RH, Van Houtven GL, Buckley MC, Depro BM (2000) Economic Analysis of Air Pollution Regulations: Miscellaneous Cellulose Manufacturing Industry/Industry Profile. RTI Project, 7647-001-011 http://www.noticeandcomment.com/Economic-Analysis-of-Air-Pollution-Regulations-Miscellaneous-Cellulose-Manufacturing-Industry-Industry-fn-56861.aspx

Bishnoi SW, Rozell CJ, Levin CS, Gheith MK, Johnson BR, Johnson DH, Halas NJ (2006) Nano Lett 6:1687–1692

Cañas A, Ariza MJ, Benavente J (2002) J Colloid Interf Sci 246:150–156

Crosland MP (1978) Gay-Lussac: Scientist and Bourgeois, Cambridge University Press, Cambridge

Evans CC (1964) Characterization of cellophane as an ionic barrier. Chemistry Research Department, U.S. Naval Ordnance Laboratory, Maryland, US Patent AD 437997

Foster LS, Gruntfest IJ (1937) J Chem Edu 14:274-276

Horcas I, Fernandez R, Gomez-Rodriguez JM, Colchero J, Gomez-Herrero J, Baro AM (2007) Rev Sci Instrum 78:013705

Je C-H, Kim KJ (2004) Sensor Actuat A-Phys 112:107-115

Kuswandi B, Jayus, Larasati TS, Abdullah A, Heng LY (2012) Food Anal Method 5:881-889

Laity PR, Glover PM, Godward J, McDonald PJ, Hay JN (2000) Cellulose 7:227-246

Naksuriya O, Okonogia S, Schiffelers RM, Hennink WE (2014) Biomaterials 35:3365-3383

Pourezza N, Golmohammadi H (2015) Talanta 131: 136-141

Proniewicz LM, Paluszkiewicz C, Weselucha-Birczynska A, Majcherczyk H, Baranski A, Konieczna A (2011) J Mol Struct 596:163-169

Salem M, Rohani S, Gillies ER (2014) RSC Adv 4:10815–10829

Sigel H, Zuberbühler AD, Yamauchi O (1991) Anal Chim Acta 255:63-72

Soni H, Patel SS, Mishra K, Nayak G, Singhai AK (2011) IRJP 2:180-184

Stock JT (1991) Bull Hist Chem 10:31-34

Szabadváry F, Oesper RE (1964) J Chem Educ 41:105-107

Thaloor D, Miller KJ, Gephart J, Mitchell PO, Pavlath GK (1999) Am J Physiol 277:C320-329

Tomé LC, Goncalves CMB, Boaventura M, Brandão L, Mendes AM, Silvestre AJD, Neto CP, Gandini A, Freire CSR, Marrucho IM (2011) Carbohyd Polym 83:836–842

U.S. Department of Agriculture, Agricultural Research Service – USDA (2004). USDA National Nutrient Database for Standard Reference, Release 17. Nutrient Data Laboratory http://www.ars.usda.gov/nuteintdata

Zhang X, Lu S, Chen X (2014) Sensor Actuat B-Chem 198:268–273

Figure Captions

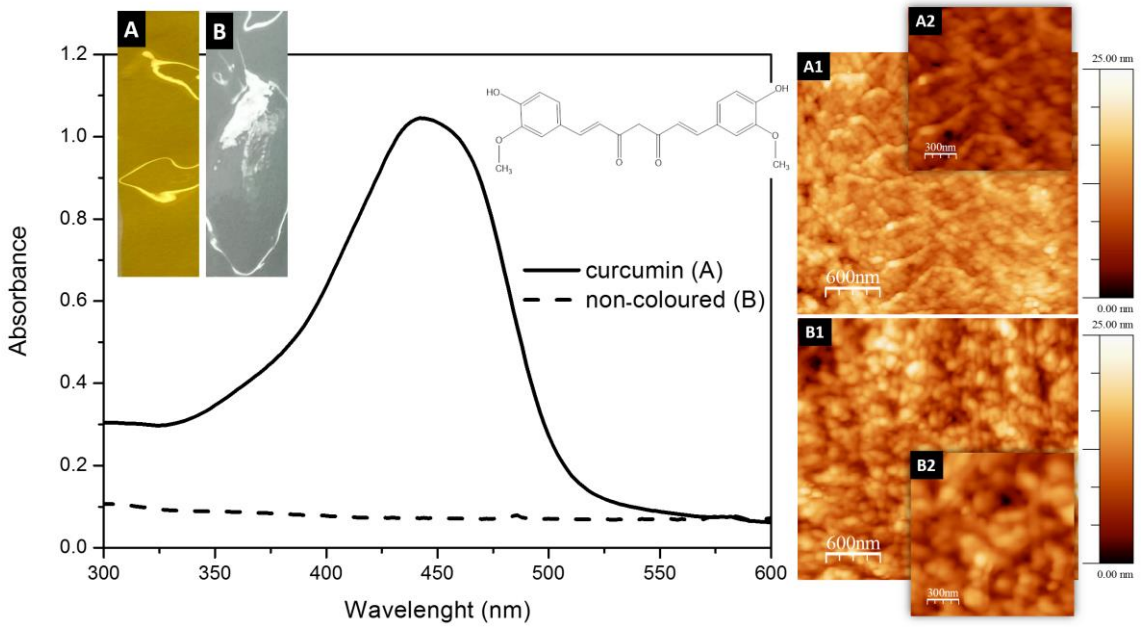

Fig. 1. Curcumin coloured cellophane test strip: Left - Absorption spectra of the curcumin coloured (A) and non-coloured test strip (B) with the photos of the test strip and the chemical structure of curcumin; Right - AFM image of the curcumin coloured (A) and non-coloured test strip (B)

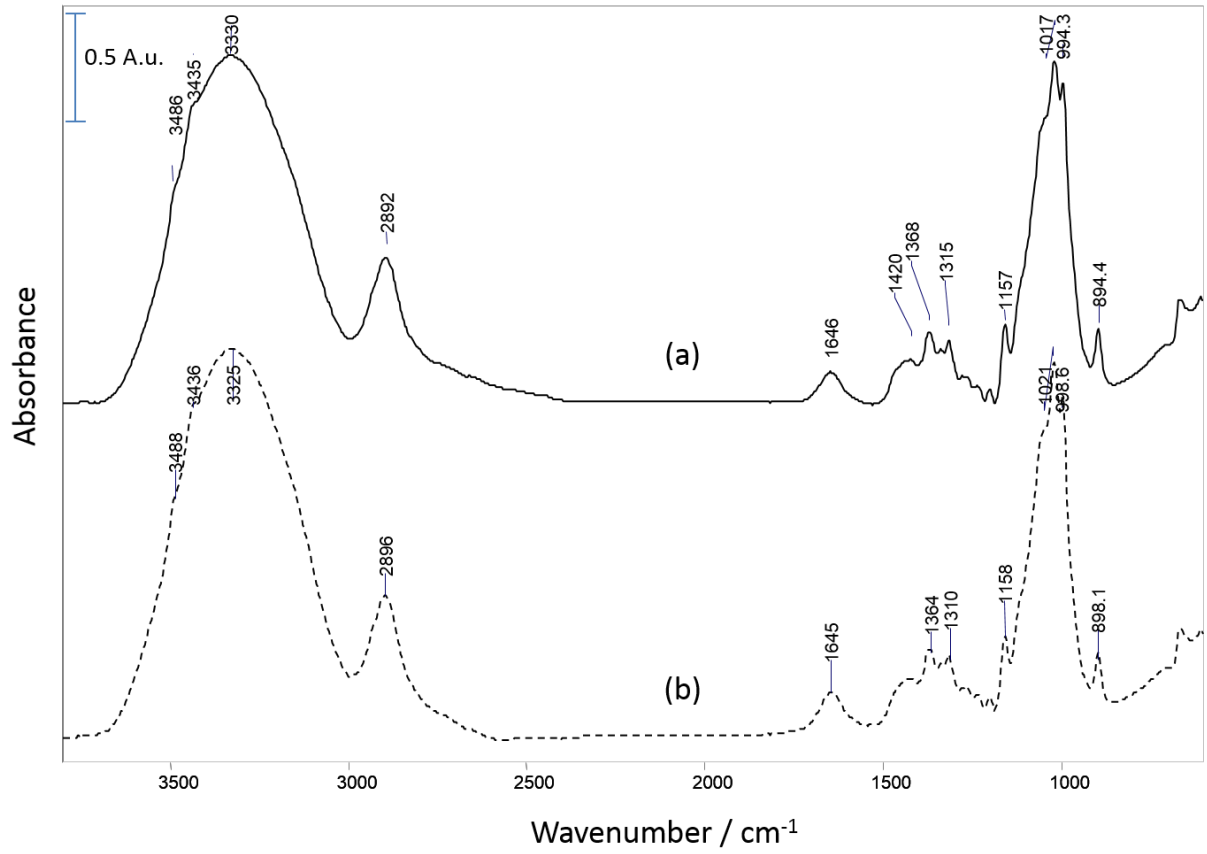

Fig. 2. FTIR spectrum of curcumin impregnated cellophane test strip (a), compared with the spectrum of pure cellophane (immersed in distilled water) (b)

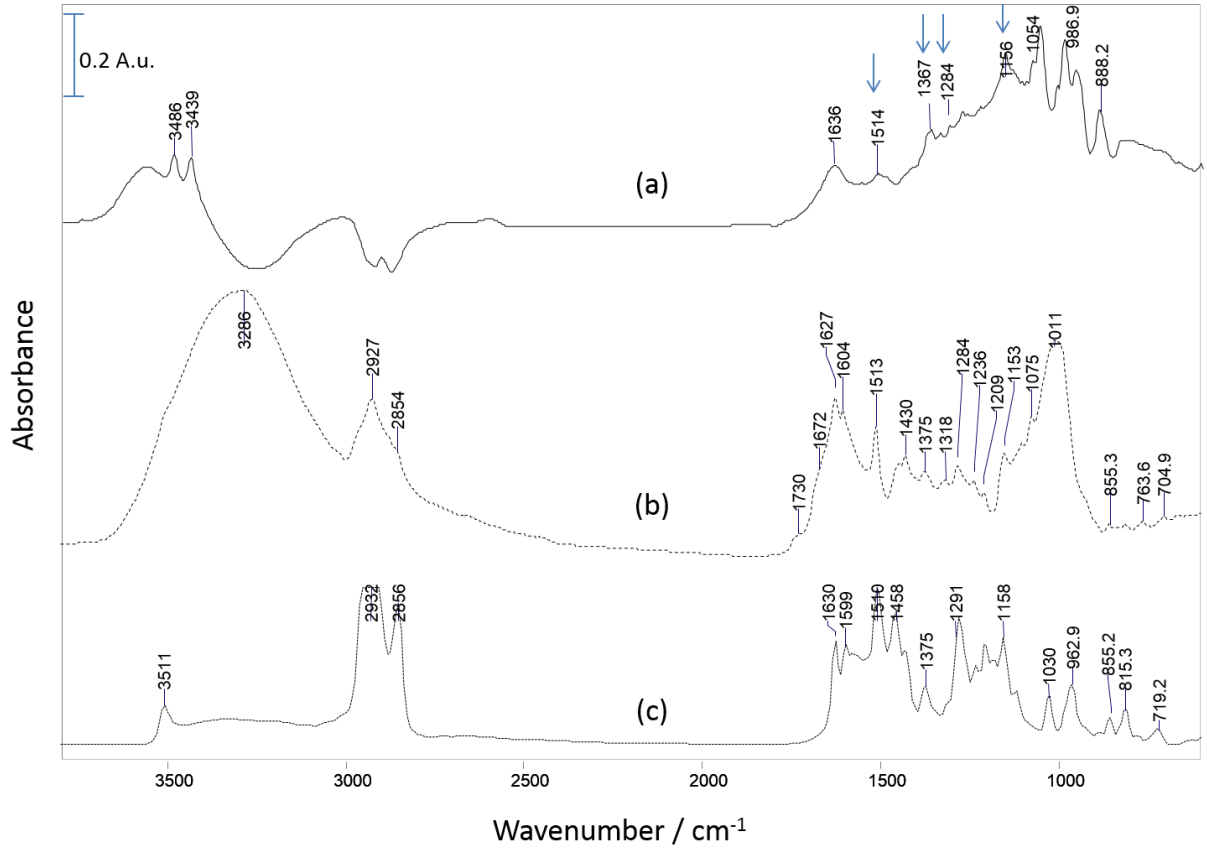

Fig. 3. Subtracted spectrum: curcumin impregnated test strip – test strip (a) compared with the FTIR spectra of curcuma powder (b) and pure curcumin reference (Aldrich FTIR Spectral Library) (c). Arrows indicate curcumin traces in subtracted spectrum.

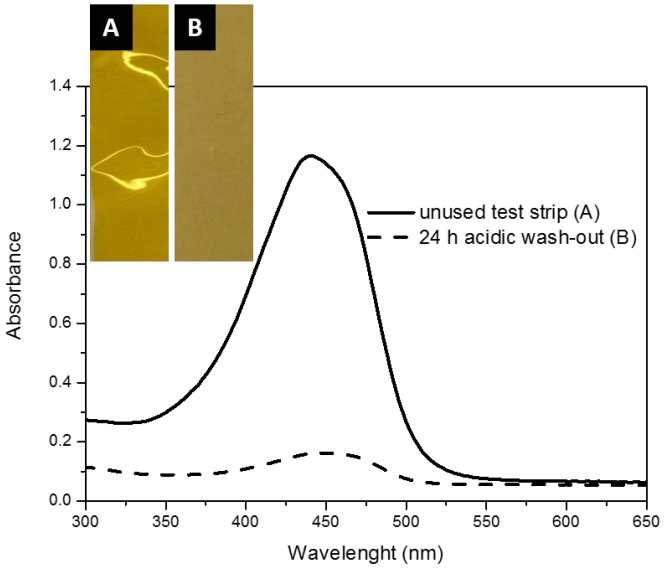

Fig. 4. Test strip stability in acidic environment: Absorbance spectra of unused (A) and treated (B - acidic acid 24 hours) curcumin coloured cellophane

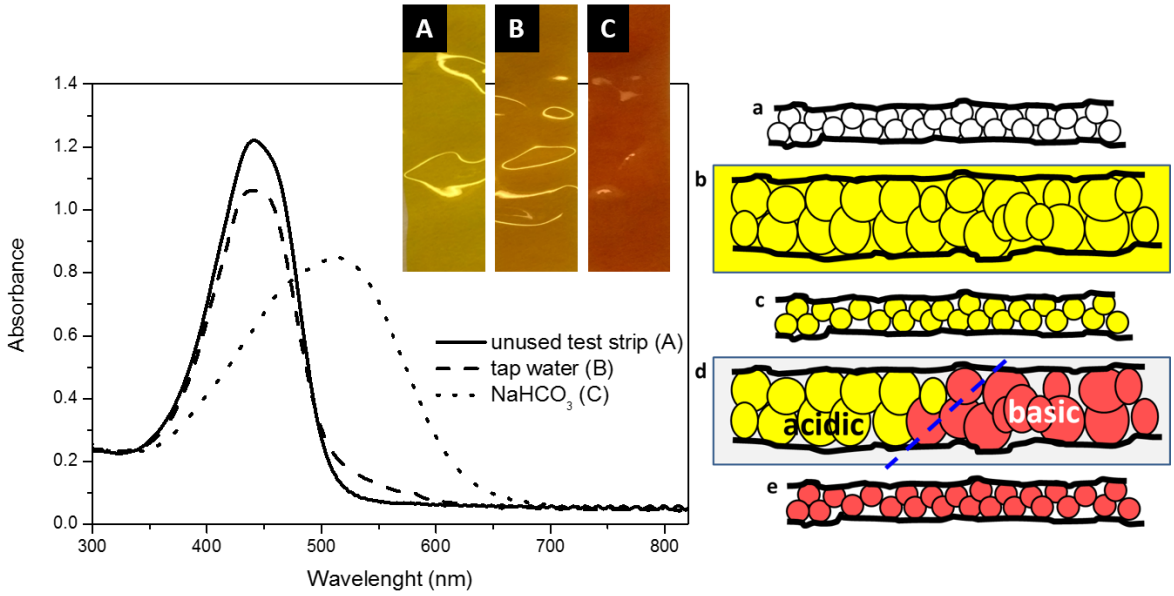

Fig. 5. Indicator effect of curcumin coloured test strip: Absorbance spectra of unused (A), in to tap water (B) and in to $NaHCO_3$ solution (C) immersed test strip with the model of the colouring (a-c) and indicator (d-e) effect

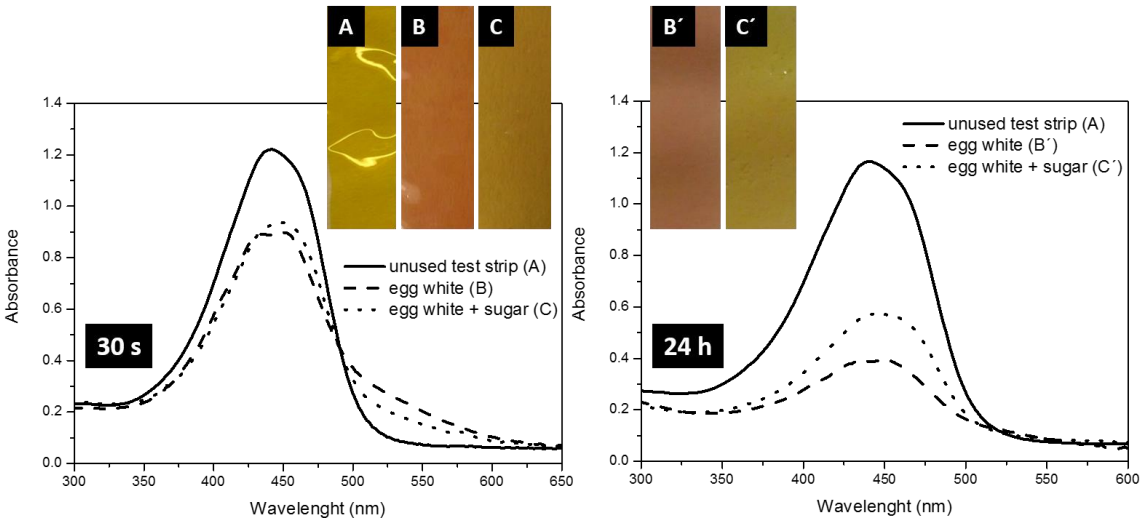

Fig. 6. Test strip (A) colour-change (30 seconds and 24 hours) in egg white (B, B') and in the presence of sugar (C, C')

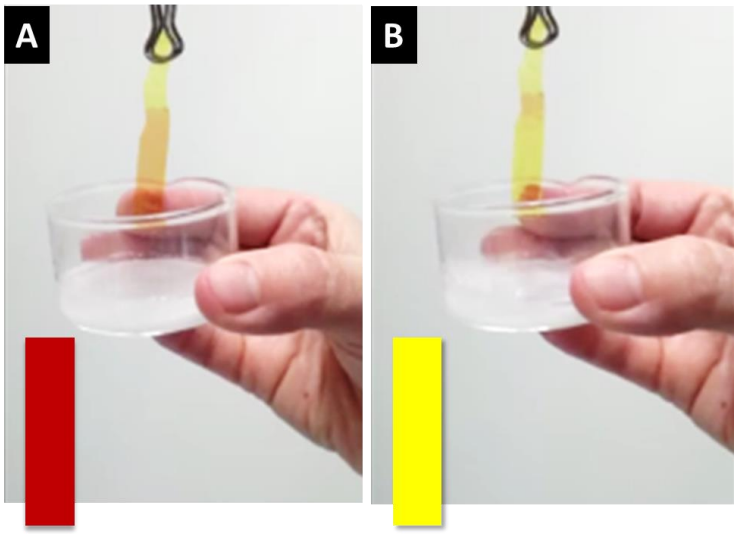

Fig. 7. Gas sensor application of the curcumin coloured test strip in CO_2 environment (A – initial step; B – colour change after some seconds)

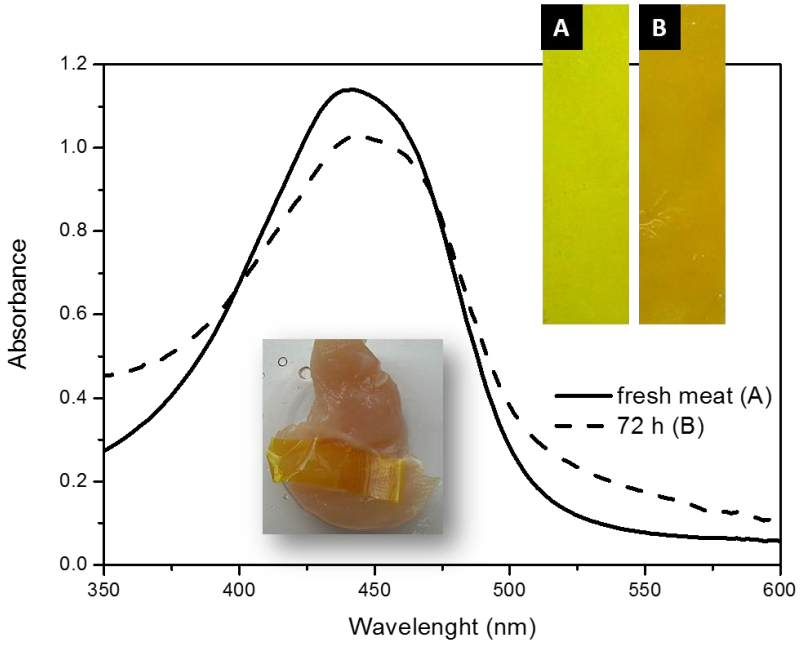

Fig. 8. Quality monitoring of raw meat: Absorbance spectra of the test strips contacted with fresh (A) and rotten (B) meat

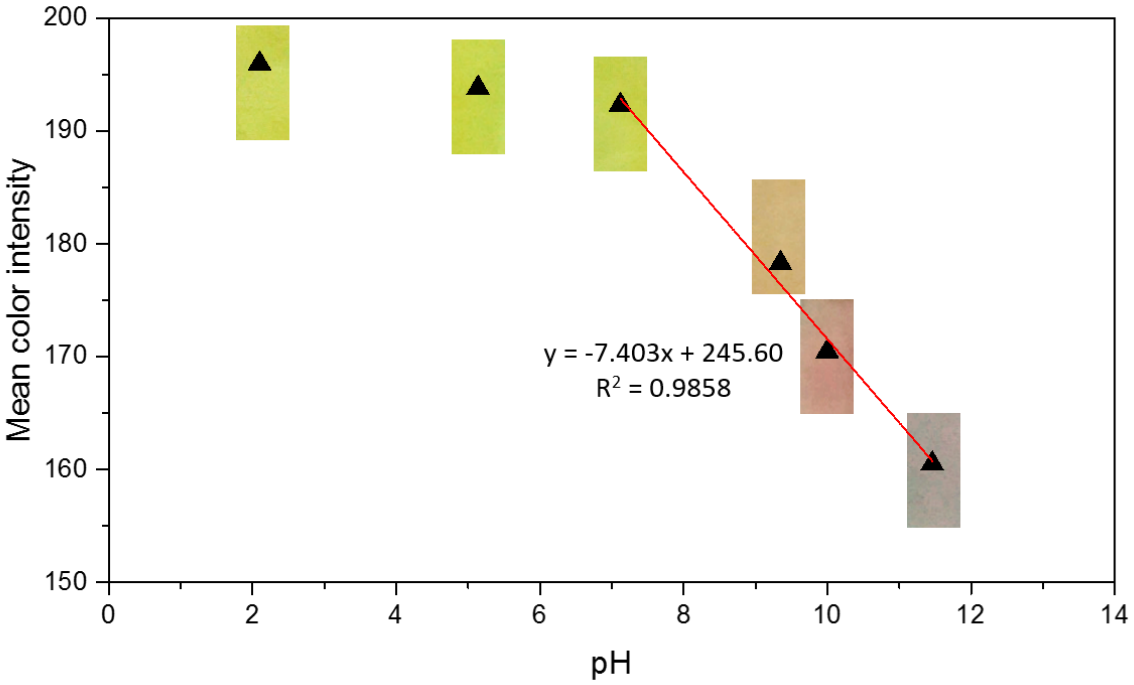

Fig. 9. Colour changes of the test strips and the corresponding calibration graph for pH sensing using curcumin coloured cellophane strip