Supporting Information

Furfural Determination with Disposable Polymer Films and Smartphone-Based Colorimetry for Beer Freshness Assessment

Alberto Rico-Yuste[$§], Victoria González-Vallejo[$§], Elena Benito-Peña[§]*, Tomás de las Casas Engel[†], Guillermo Orellana[†], María Cruz Moreno-Bondi[§]*

[§]*Department of Analytical Chemistry and* [†]*Department of Organic Chemistry, Faculty of Chemistry, Complutense University, Ciudad Universitaria s/n, Madrid 28040 (Spain),*

**Corresponding authors: María Cruz Moreno-Bondi and Elena Benito-Peña., Department of Analytical Chemistry, Faculty of Chemistry, Universidad Complutense de Madrid, Avenida Complutense, s/n Madrid, 28040, Spain. E-mail: mcmbondi@ucm.es and elenabp@ucm.es, Tel: +34-91394-5147, Fax: 34-91 394 4329*

[$]*Authors equally contributed to this work.*

Content Index

1. Volatile aldehydes generated during beer aging

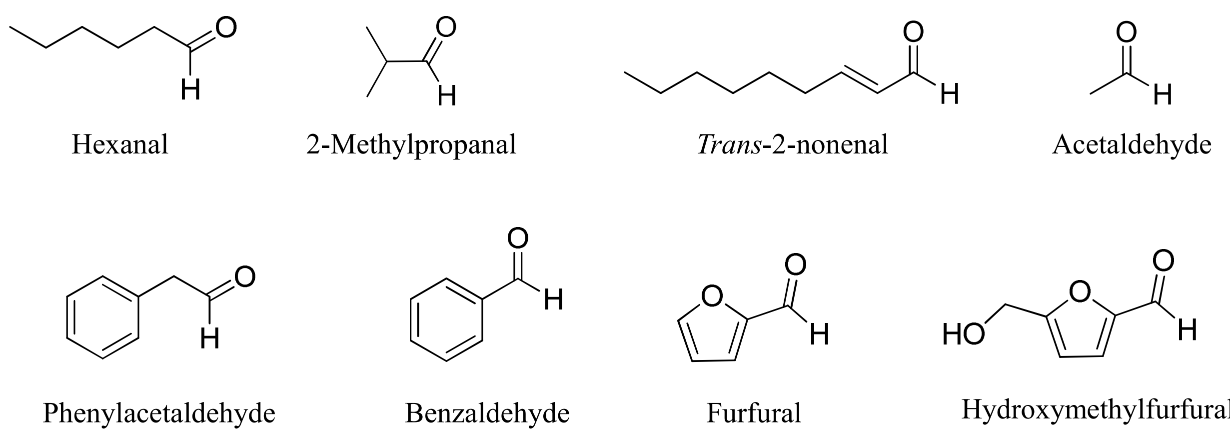
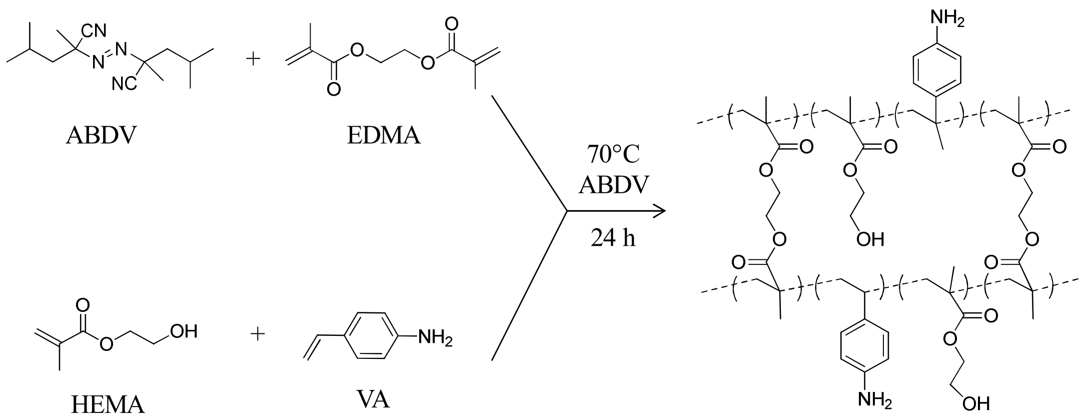


Figure S1. Typical volatile aldehydes produced during the beer ageing process.

2. Fabrication and optimization of membrane composition



Scheme S1. *Scheme of the polymer synthesis.*

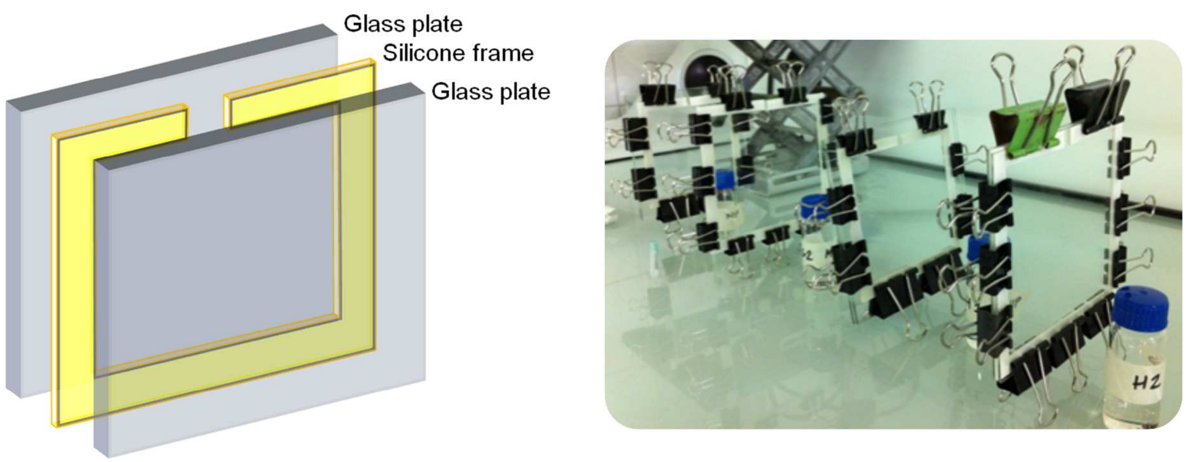


Figure S2. *Scheme of the polymerization molds used for the films preparation. The glass slides (10 × 10 cm) were separated by a 0.9 mm thick silicone gasket (1 cm wide), compressed to create a leak-free seal and filled in with the prepolymerization mixture.*

3. Synthesis of furfural-responsive polymer films

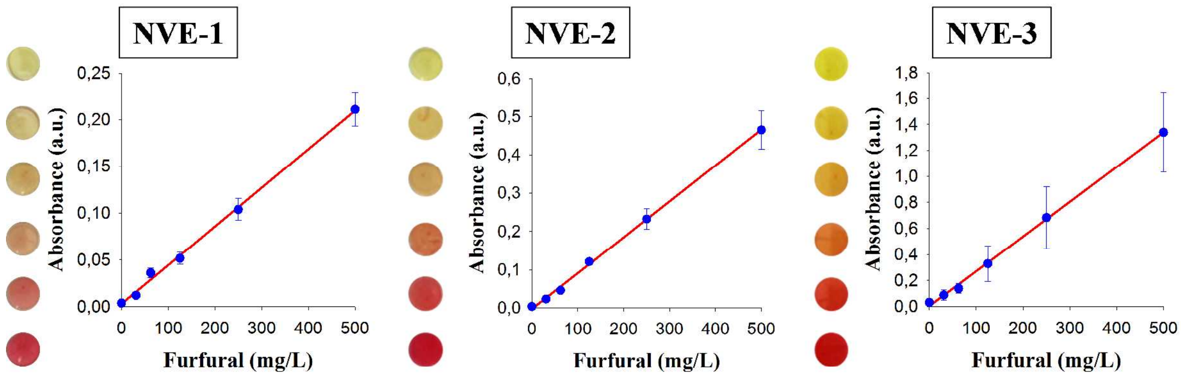


Figure S3. *Representative calibration plots (*n *= 3) obtained with the sensor disks in 10 mL* water/ethanol (80/20, *v/v*) *solutions spiked with furfural at (●) 0, (●) 31, (●) 63, (●) 125, (●) 250 and (●) 500 µg L*$^{-1}$ *(*λ_{abs} *= 537 nm).*

4. Effect of the membrane thickness on the sensor response

The effect of the film thickness on the homogeneity of the colorimetric sensor was evaluated by incubating 0.5- and 0.9-mm thick disks for 1 h in 10 mL samples of 500 µg L-1 furfural in water-ethanol (80/20, *v/v*). As observed in Figure S4, the 0.9 cm-thick film showed an inhomogeneous response, likely due to the slower diffusion of the analyte through the thicker layer resulting in an incomplete reaction within the incubation time (1 h). This effect was not observed with the 0.5 cm-thick sensing discs that showed a uniform response throughout the surface and were selected for further experiments.

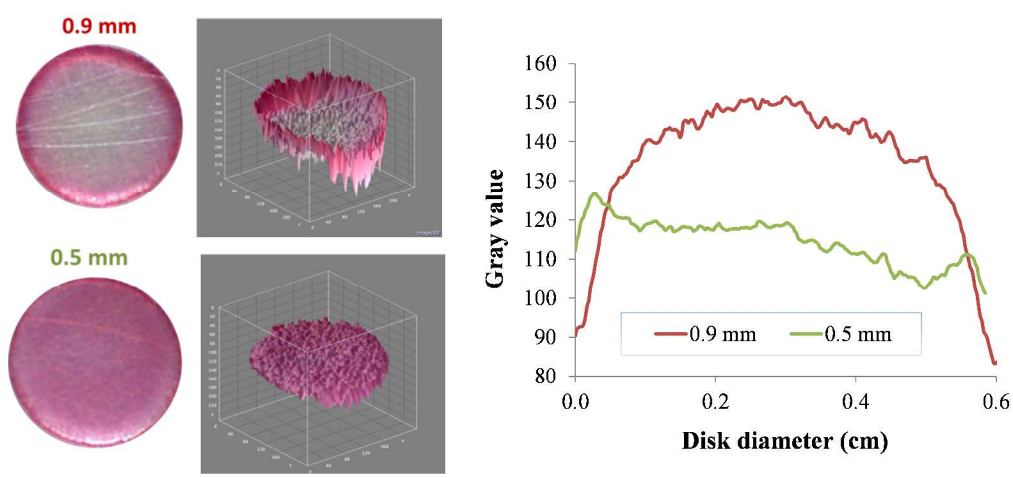


Figure S4. *Effect of membrane thickness on the sensor response to 500 µg L*$^{-1}$ *furfural in water-ethanol (80/20, v/v). Left, 3D image of the surface. Right, gray value variation along the disk diameter for a 0.9 and a 0.5 cm membrane thickness.*

5. Effect of the acid catalyst concentration on the sensor response

The Stenhouse reaction between aniline and furfural takes place in acidic media. Therefore, the effect of the acid concentration in the measuring sample on the sensor response was studied by immersing the sensing disks in 10 mL samples containing 500 µg L^{-1} of furfural in water-ethanol (80/20, *v/v*) spiked with HCl at different final concentrations (5, 29, 49 and 216 mM). All the experiments were done in triplicate. As observed in Figure S5, the sensitivity of the assay increased with the concentration of acid in the sample, reaching a maximum at 29 mM HCl. Higher concentrations resulted in lower sensitivities and membrane damage as observed in 216 mM HCl. It has been described in the literature that swelling of polyHEMA hydrogels is higher in basic than in acidic medium,[1] which would explain the sensitivity lost at higher HCl concentrations. However, the Stenhouse reaction requires the presence of an acidic catalyst so that finally a 29 mM HCl concentration in the sample was selected for further assays.

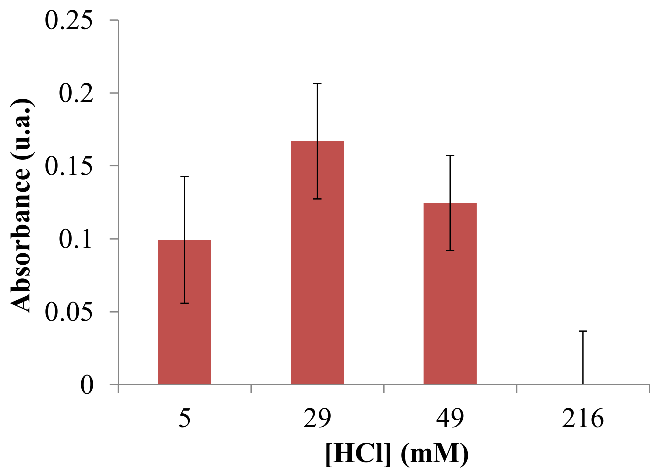
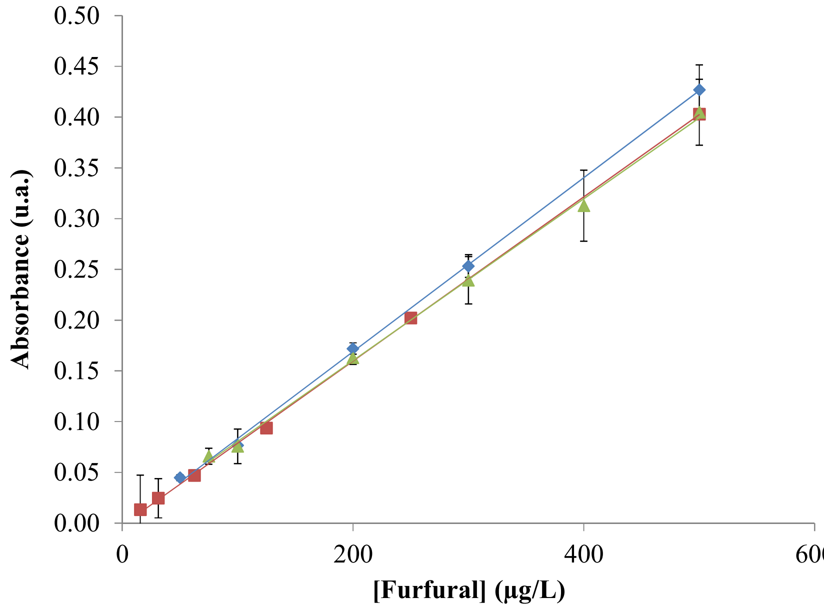


Figure S5. *Effect of the HCl concentration in the sample on the sensor response. [Furfural] = 500 µg L^{-1} (*n = *3; λ_{abs} = 537 nm).*

6. Effect of the sample ethanol content on the sensor response



(◆) 0.0 % EtOH:	A (a.u.) = 858 (14) $\times 10^{-6}$ [Furfural] + 30 (40) $\times 10^{-4}$	$r = 0.9996$
(■) 5.4 % EtOH:	A (a.u.) = 810 (8) $\times 10^{-6}$ [Furfural] + 22 (19) $\times 10^{-4}$	$r = 0.9998$
(▲) 20 % EtOH:	A (a.u.) = 800 (20) $\times 10^{-6}$ [Furfural] + 10 (50) $\times 10^{-4}$	$r = 0.9992$

Figure S6. *Effect of the ethanol concentration in the standard solutions of furfural (0 - 500 µg L^{-1}) on the sensor response: (◆) 0.0 %, (■) 5.4 % y (▲) 20 % (v/v)* (n = 3; λ_{abs} = 537 nm). *The values in parentheses indicate the standard deviations (SD).*

7. Reproducibility of the disks fabrication

To investigate the reproducibility of the sensing film fabrication, three different batches were prepared in different months by independent operators. The sensors response to furfural standards (200 and 500 µg L^{-1}) prepared in water-ethanol mixtures (94.6/5.4, *v/v*) are depicted in Figure S7. All the experiments were done in triplicate. No significant differences were observed (at a 95% confidence level) between the different batches (RSD ≤ 11%), demonstrating the reproducibility in the preparation of the sensing films.

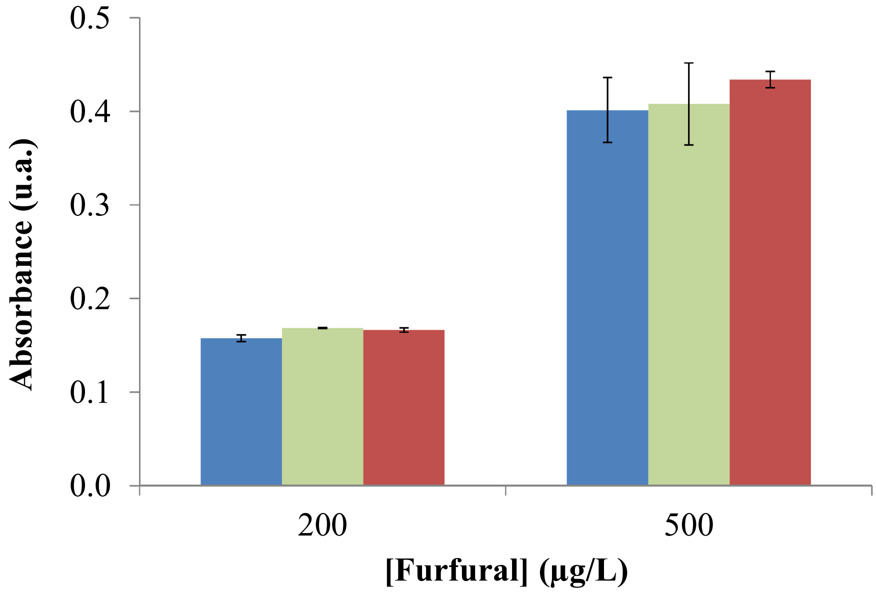


Figure S7. *Evaluation of the reproducibility of the sensing film fabrication: ( ) Month 1, ( ) Month 3 and, ( ) Month 4 (*n = 3; λ_{abs} = 537 nm*). Incubation solvent: water-ethanol (94.6/5.4, v/v)*

8. Sensor validation

The sensors were applied to the analysis of furfural in water-ethanol (94.6/5.4, *v/v*) samples spiked with furfural at six concentration levels (75 - 500 µg L^{-1}) and the results have been validated by HPLC-DAD.

The results, collected in **Error! Not a valid bookmark self-reference.**, showed no significant differences (confidence level 95%, n = 3) between both methods at any of the furfural concentration levels tested.

Table S1. *Fiber-optic based sensor validation by HPLC-DAD (n = 3).*

	[Furfural] (µg L^{-1})	
Spiking	**Found *(Fiber-optic spectrophotometer)***	**Found *(HPLC-DAD)***
75	77 (10)	75 (1)
100	100 (20)	99.0 (0.1)
200	200 (6)	200 (2)
300	300 (23)	301.3 (0.4)
400	402 (16)	400 (2)
500	495 (40)	500 (2)

(SD) = Standard deviation

9. Evaluation of the matrix effects in real samples

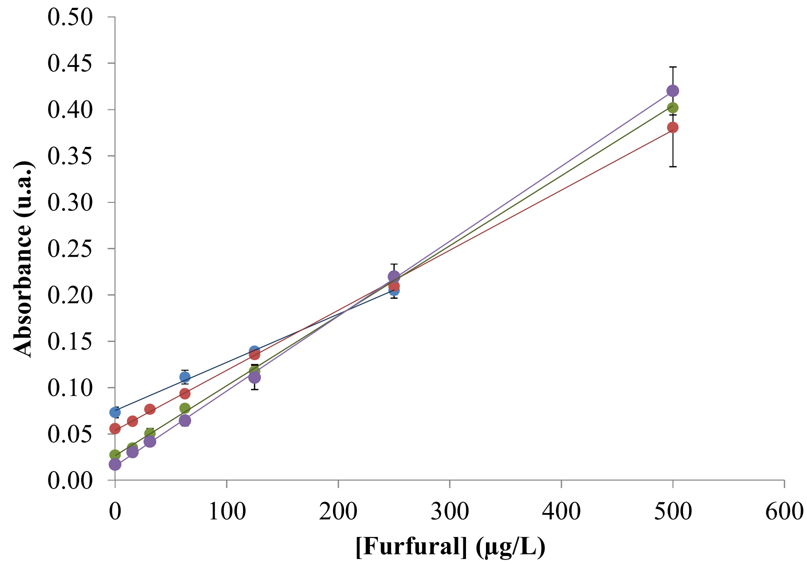


Figure S8. *Evaluation of the matrix effect on the sensor response. Sample solutions: (●) water-ethanol (94.6/5.4, v/v), (●) beer, (●) beer diluted (1:1) with water-ethanol (94.6/5.4; v/v) and, (●) beer diluted (1:4) with water-ethanol (94.6/5.4, v/v). [Furfural]: 0 – 500 µg L^{-1}* (n = 5; λ_{abs} = 537 nm).

10. Linear correlation between storage time and furfural content

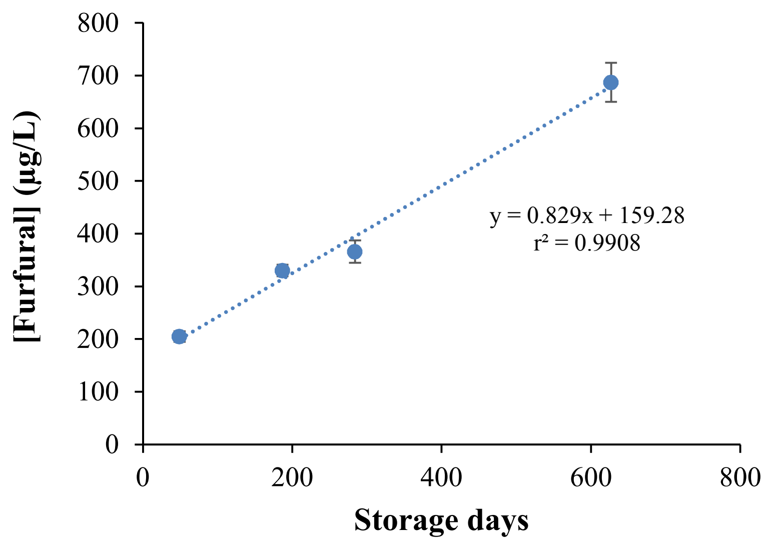


Figure S9. *Correlation between the furfural concentrations in the beer samples, measured with the colorimetric sensors, and the days of storage. Linear correlation is confirmed by the r-value (0.995).*

11. References

(1) Gómez, M. L., Williams, R. J. J., Montejano, H. A., Previtali, C. M. *eXPRESS Polym. Let.* **2012**, *3*, 189-197.