Supplementary Information

Colorimetric paper bioassay for the detection of phenolic compounds

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Quantification of color and calibration of the paper sensor

A HP Scanjet 4070 Photosmart Scanner was used to scan the spherical disks of the colorimetric paper sensor after exposure to phenols, at a resolution of 2400 dots per inch (dpi). Quantification of the color intensity was made using Adobe Photoshop (version 7.0). The eyedropper tool in this software was used to quantify the color intensity based on the color wheel chart. For example, the blue color derivatives are the complementary colors (*i.e.* negative images) of orange-brownish, while the greenish color derivatives are complementary colors of pink-reddish colors. The reaction of phenol, catechol, and cresols with the chitosan-tyrosinase modified paper formed an orange-brownish color onto the sensor surface. Therefore, the blue color intensity was used for quantification, as the complementary color provided higher sensitivity than the other colors of the red-green-blue (RGB) color mode in Adobe Photoshop. Furthermore, since BPA formed a green color onto the disk surface, a reddish complementary color was used in this case, which provided a higher sensitivity for BPA in RGB color mode.

UV-VIS spectroscopy study

UV-VIS measurements were carried out using a Shimadzu UV-VIS spectrophotometer (model Shimadzu UV-2401 PC) equipped with a 1-cm path length cell. The activity of the tyrosinase was measured using a colorimetric assay according to established procedures.[1] UV-VIS measurements were performed by measuring the spectrum of 0.1 mM phenolic compound added to a solution of chitosan at a concentration of 0.125 % (w/v), in the presence and absence of Tyrosinase (1500 U/ml). The progression of the reaction was monitored over time until a stable spectrum was obtained. Spectra were recorded at time 0 and then at 10 min, 60 min, 120 min, 240 min and at 24 hrs interval.

For both phenol and catechol, a peak with a maximum at ~480 nm appeared in presence of chitosan. A less intense peak at the same wavelength was observed for *m*- and *p*-cresol. Appearance of the color was almost instantaneous for simple substituted phenols. The reaction proceeded fast and was completed within 2 hrs for catechol, phenol and cresols. Among these, the faster reaction time was observed for catechol, an o-diphenol which is known to undergo rapid two-electron oxidation to o-quinone through

the catecholase activity of tyrosinase,[2] while for phenol, both cresolase and catecholase activities are involved in the biocatalytic conversion.

For BPA which is comprised of two joined phenolic rings, the reaction was noticeably slower. A blue/green color started to appear after about 10 min and progressed overtime reaching a steady-state level after 24 hrs. BPA has a well-defined absorption maximum at 224 nm and a small peak at 276 nm. The two peaks increase in the presence of tyrosinase, concomitant with the appearance of a new peak at ~390 nm, corresponding to the formation of a quinoid compound.[3] When chitosan is added to a solution of BPA and tyrosinase, a new well-defined peak was observed at ~610 nm which is responsible for the formation of the green-blue color. The slow kinetic and the characteristic blue/green color indicate a change in the optical properties and the degree of conjugation as compared to single phenols, which might be due to the rigidity of the structure and the multiple-step enzymatic process involving conversion of each of the two phenolic groups to form catechols, followed by formation of quinone-like structures at the two rings followed by binding to chitosan. Interestingly, when a structurally similar compound, diethylstilbestrol (containing two phenolic rings but joined by a double bound with di-ethyl groups) was tested, no color change was observed in the presence of chitosan. It is possible that the flexibility of the structure and the side di-ethyl substituents could cause steric hindrance and prevent further binding of the highly reactive diethylstilbestrol quinone to chitosan.

In an attempt to expand use of this assay to other phenolic compounds and establish a structure-activity relationship we have tested several other phenolics that are known as tyrosinase substrates, able to form o-substituted quinones. The compounds tested include dopamine, a catecholamine with a catechol linked structure, as well as more complex polyphenols like quercetin (3,3',4',5,7–pentahydroxyflavanone) containing a catechol moiety linked to a condensed flavone backbone (3-phenyl-1,4-benzopyrone) and resveratrol. For dopamine, a fast reaction kinetic, similar to that of catechol was observed, resulting in a dark brown color with an absorption maximum at ~480 nm in presence of chitosan. No color was observed for both quercetin and resveratrol, although both polyphenols are known as being enzymatically converted to quinones.[3] It is possible that conformational constraints due to the presence of bulky aryl

substituents (e.g. flavone ring in quercetin) as well as the conformational flexibility (e.g. resveratrol) restrict binding of the generated quinone to chitosan.

These results indicate that the chemical structure of the phenolic compounds, e.g. presence and nature of different substituents onto the phenolic ring, affect the reaction kinetic and mechanism as well as determine the spectral features of the quinone-chitosan complexes. The structure, specifically the rigidity, and number and type of substituents affect both the enzymatic reaction with formation of quinone-type compounds, as well as their subsequent binding to chitosan. Consequently, these parameters are important considerations in utilizing these properties for designing quantitative analytical bioassays, such as the tyrosinase-based paper sensor described in this work.

Development of the color intensity over time.

Figure S1 below shows development of the color over time after addition of 100 μg/L phenol (A) or BPA (B). Images were taken every 30 sec for phenol and 60 sec for BPA using an iPhone camera.

Figure S1A

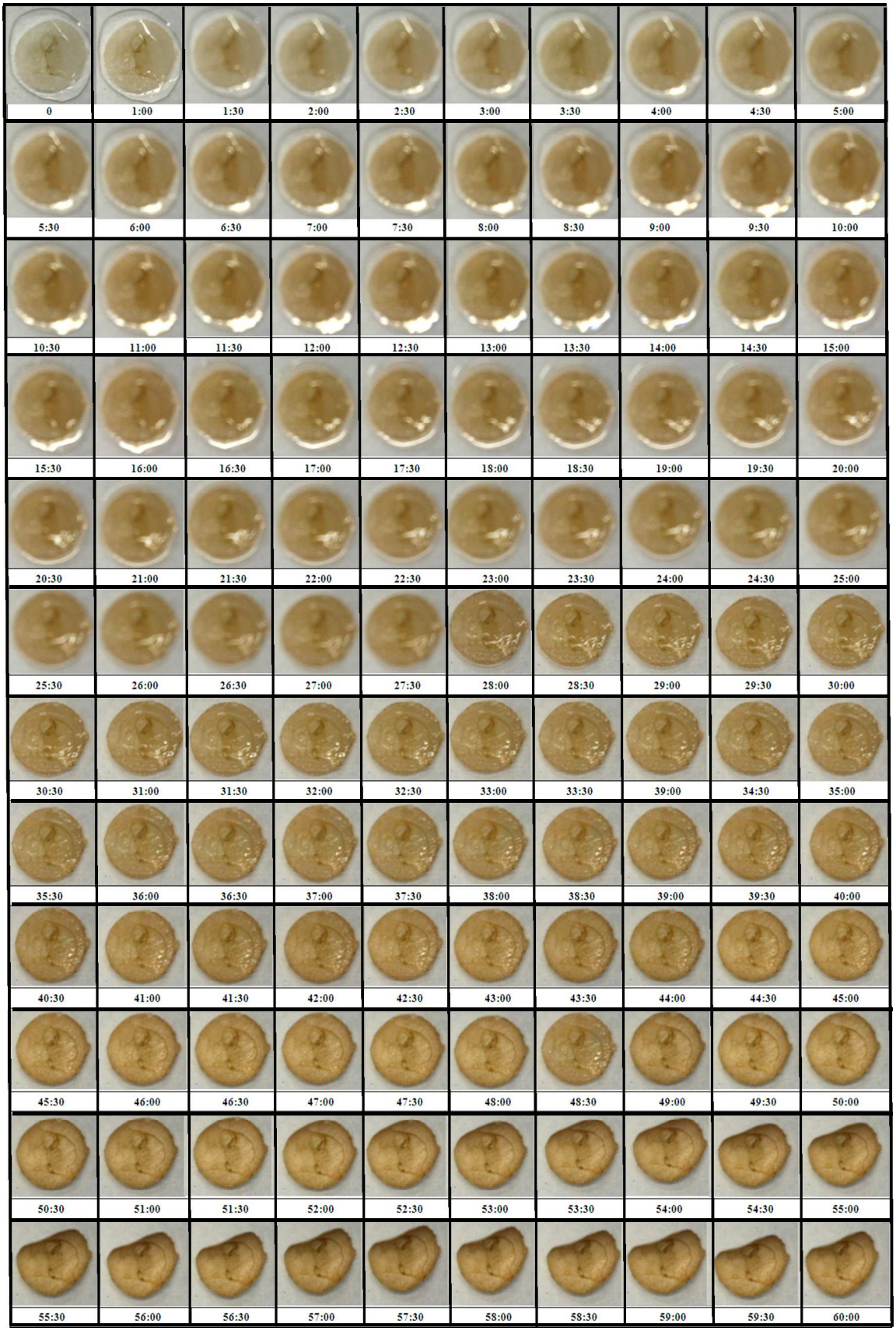
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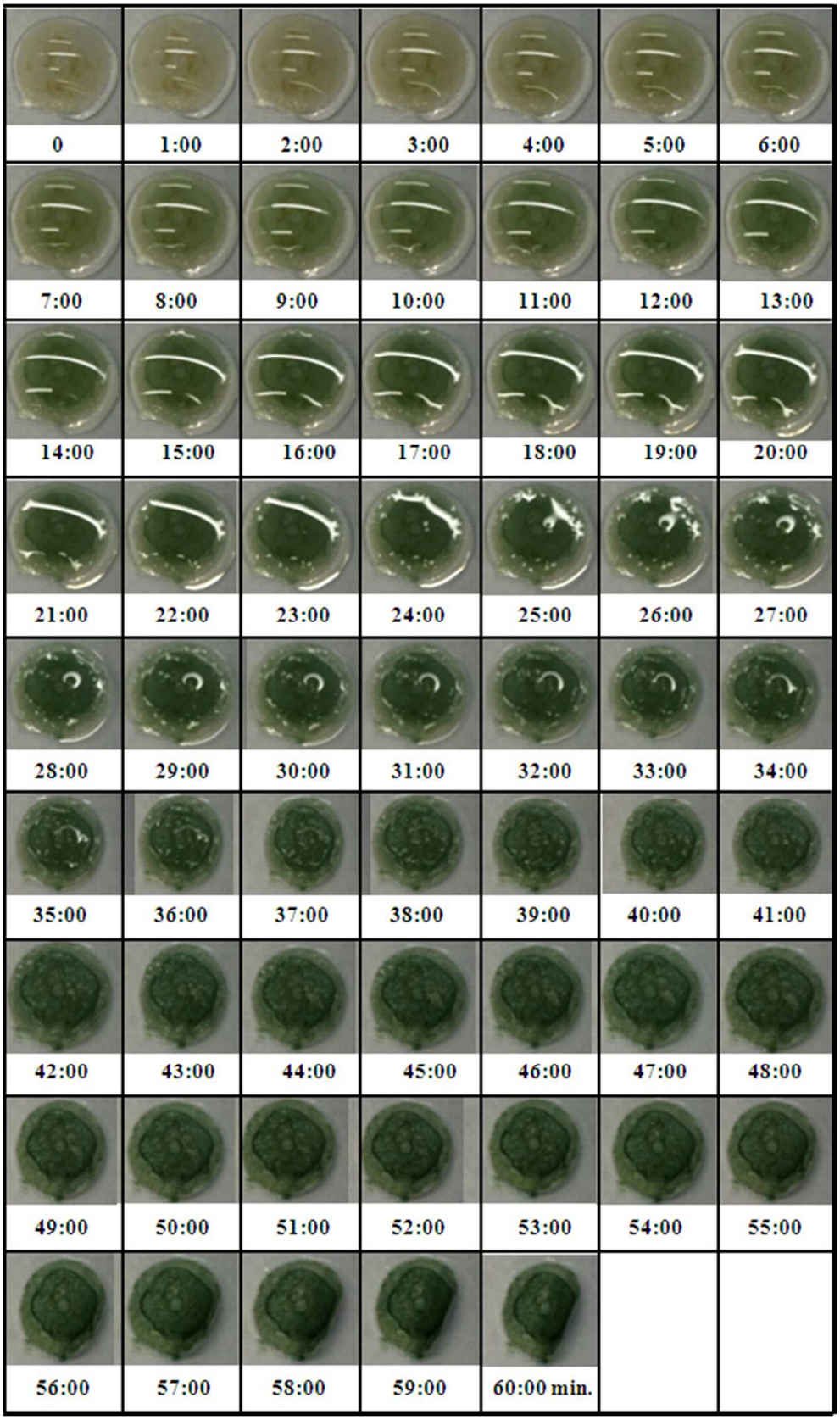
Figure S1B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Study of interferences

Figure S2 below shows the selectivity of the LbL tyrosinase sensors against potential interfering compounds: phenylananine, ascorbic acid and uric acid and the sensor selectivity to 100 µg/L phenol in mixture with ascorbic acid at various concentrations (50-250 µg/l).

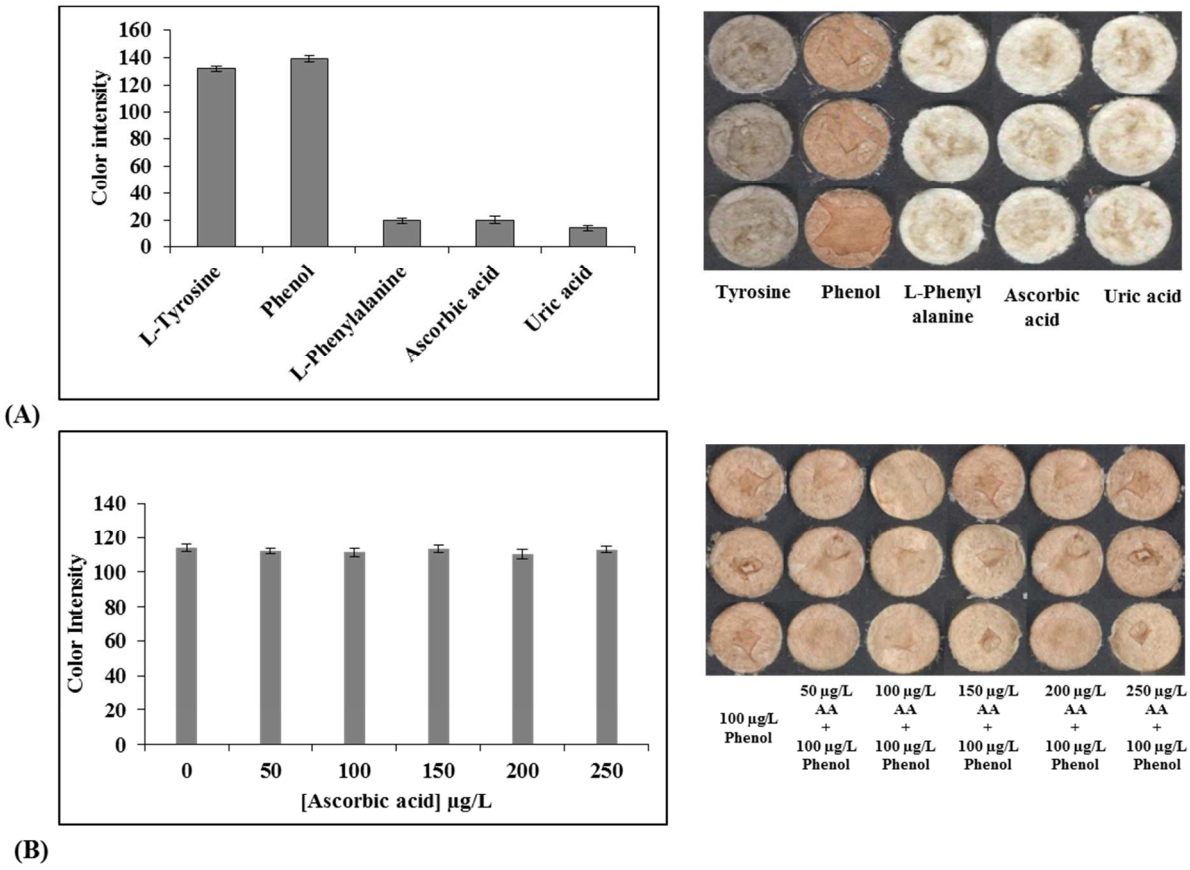

Figure S2. Selectivity of the paper sensors against potential interfering compounds (A) and sensor selectivity to 100 µg/L phenol in mixture with ascorbic acid (B). All solutions are prepared in 0.1 M PBS pH 6.5. Error bars shows mean values for $n = 3$ sensors.

Study of reproducibility

Figure S3 shows the reproducibility of the colorimetric sensors quantified by measuring the color intensity of three identical sensors after addition of 100 µg/L phenol or BPA.

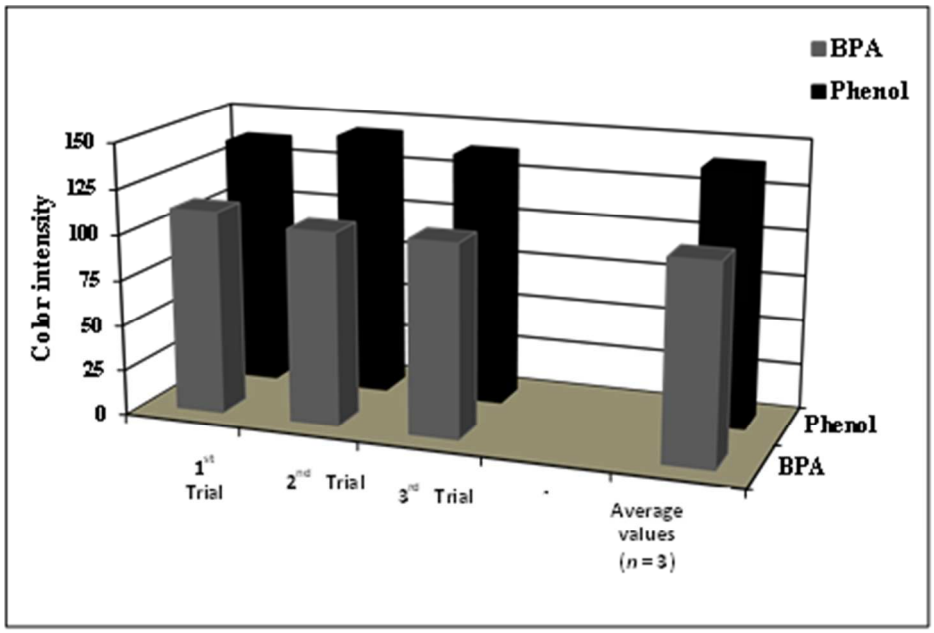

Figure S3. Reproducibility of the colorimetric sensors.

Operational stability

Figure S4A shows the storage stability histograms quantified by the color intensity of the sensors in response to BPA and phenol under the three storage conditions. A concentration of 50 µg/L BPA or phenol was used in these measurements, and all experiments were done in triplicate. Figure S4B shows the corresponding colorimetric responses under these storage conditions.

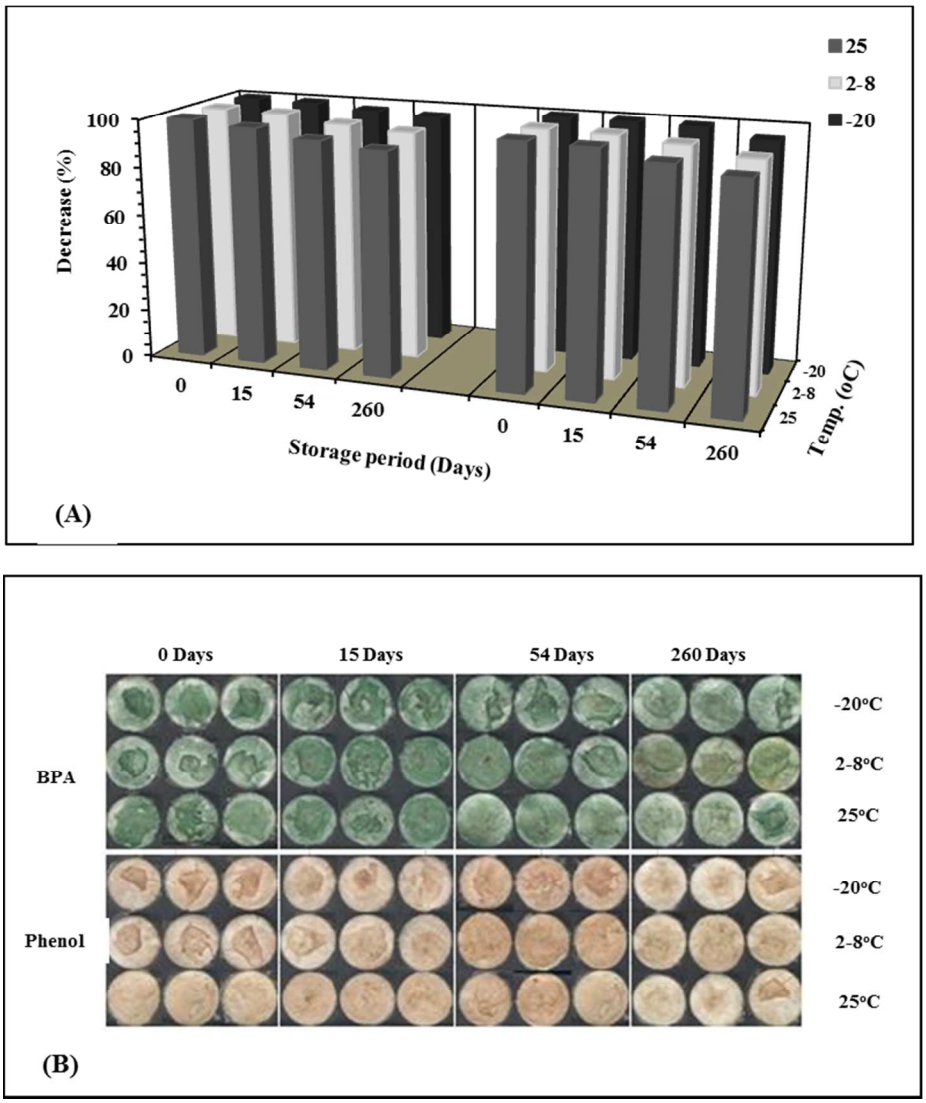

Figure S4. (A) Storage stability of the colorimetric sensors in three storage conditions: room temperature (~25 ^{o}C), refrigerator (2-8 ^{o}C) and freezer (~ -20^{o}C). Histograms show % decrease of sensor response with day 0 corresponding to 100%. Responses are quantified as the color intensity of the paper disks after addition of 50 µg/L BPA and phenol. (B) Colorimetric responses of triplicate sensors following storage in these conditions.

Real sample analysis

Table S1. Color intensity and % recovery of sensor response: application to analysis of tap and river water samples. Samples were spiked with 50 µg/L BPA.

Sample	CI	[BPA] (µg/L)	Recovery (%)
PBS buffer 50 ug/L BPA	107	50	100
Spiked Tap Water	113	48.7	97
Spiked River Water # 1	109	46.8	94
Spiked River Water # 2	111	47.8	98
CI: Color Intensity			

A **B** **C** **D**

A: Reference sample 50 µg/L BPA

B: Spiked tap water

C: Spiked River Water # 1

D: Spiked River Water # 2

Figure S5. Real sample analysis of tap and river water samples spiked with 50 µg/L BPA.

Inkjet-printed tyrosinase paper sensors

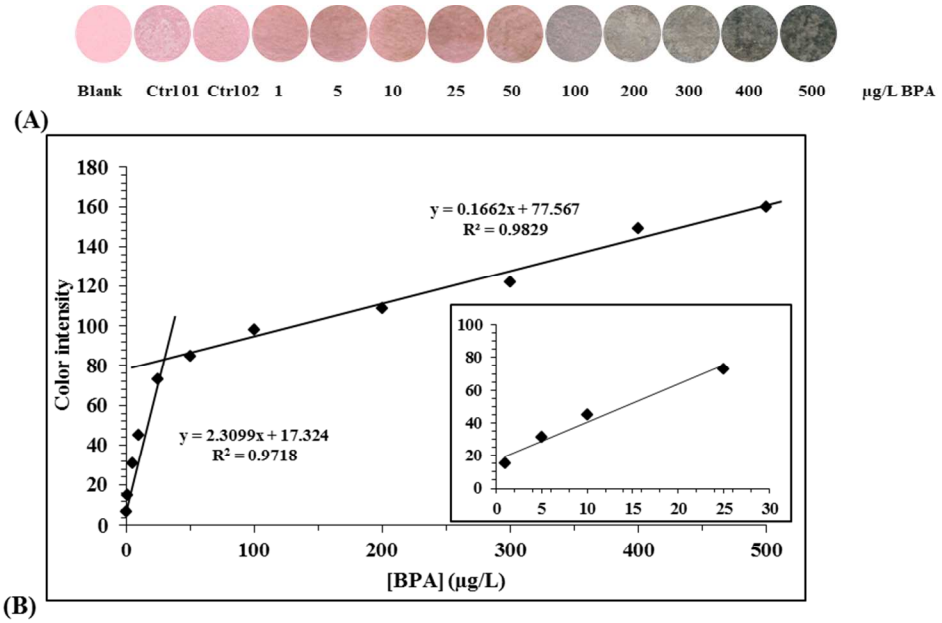

Figure S6. (A) Colorimetric response of printed sensors to additions of BPA. *Ctrl* 01: Chitosan and alginate layers without enzyme. *Ctrl* 02: Chitosan and alginate layers with enzyme. *Blank*: 0.1 M PBS pH 6.5 pink A4 sheet. (B) Calibration curve for the detection of BPA of the printed sensor (inset; the linear range). **A A4 pink-colored paper (95 µm thickness) was used as printing platform to allow visualization of the sensing spot.*

Table S2. Comparison between manually prepared and printed sensors.

BPA (µg/L)	Manual Method		Printing Method	
	Sensitivity (CI/ µg.L^{-1})	R^2	Sensitivity (CI/ µg.L^{-1})	R^2
1-25	2.8665	0.9755	2.0309	0.9718
50-500	0.2018	0.9652	0.1662	0.9829

References

(1) Worthington, v. *Worthington Enzyme manual, enzymes and related biochemicals*; Worthington Biochemical Corporation: New Jersey USA 07728, **1993**.

(2) Solomon, E. I.; Sundaram, U. M.; Machonkin, T. E. *Chem Rev* **1996**, *96*, 2563.

(3) Andreescu, S.; Sadik, O. A. *Anal Chem* **2004**, *76*, 552.