Electronic Supplementary Information (ESI)

Paper microzone assay embedded on a 3D printed support for colorimetric quantification of proteins in different biological and food samples

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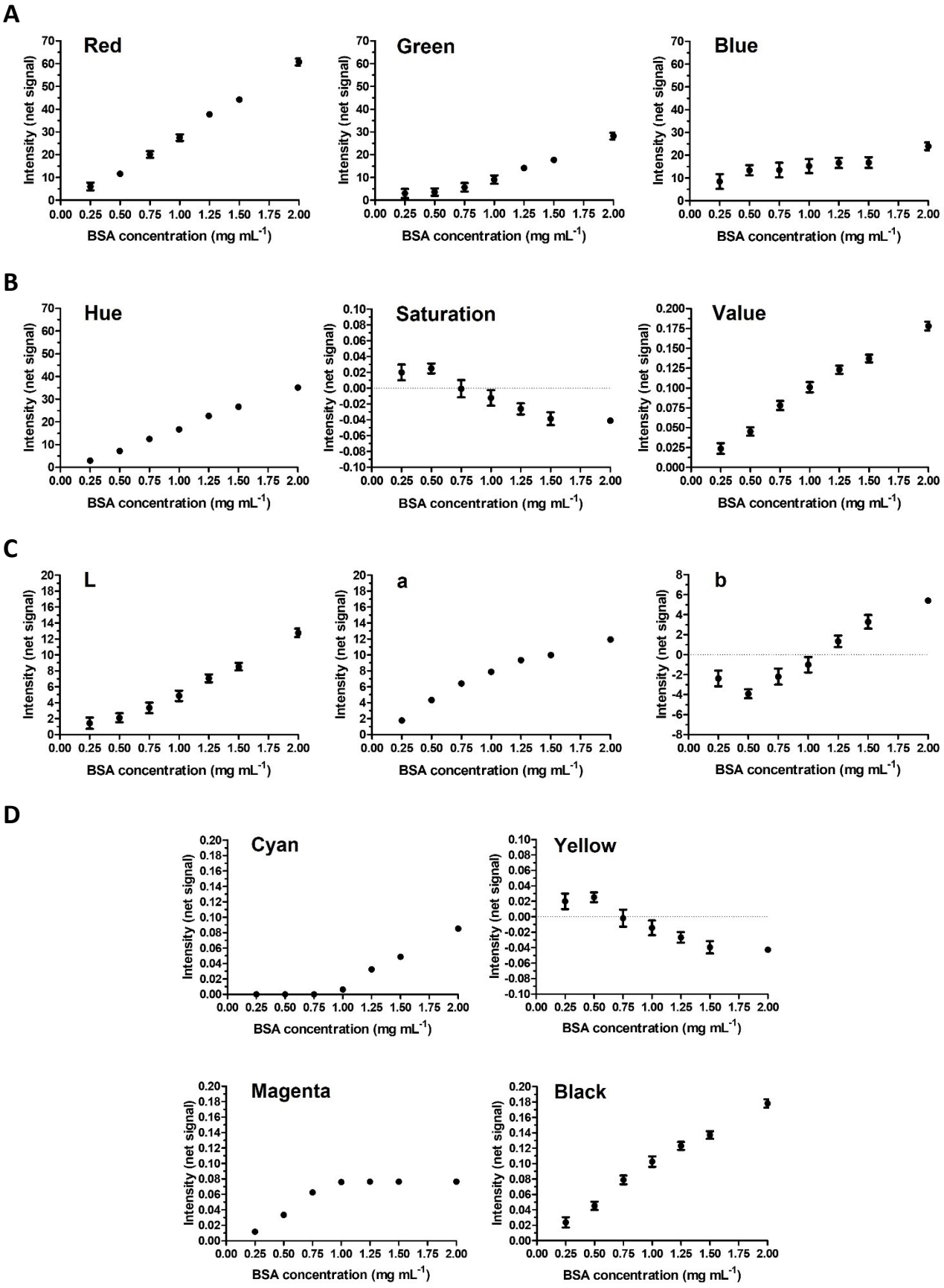

Fig. S1. Comparison of the analytical signals in the RGB (A), HSV (B), CIELAB (C) and CYMK (D) colour spaces of BSA's calibration curve from 0.25 to 2 mg ml^{-1}. The assay was performed by loading 20 µL of BPB reagent

+ BSA (10:1) and allowing the PAD to dry for 7 minutes at 50°C. Colour intensities were calculated by ColorScan software following image acquisition by a flatbed scanner. Results are expressed as mean±S.E.M. of 10 replicates. Colour intensities in CYMK colour space have been calculated as follow. The R, G, B values were divided by 255: R' = R/255, G' = G/255, B' = B/255. The black (K) colour was calculated from the red (R'), green (G') and blue (B') colours: K = 1-max(R', G', B'); the cyan color (C) was calculated from the red (R') and black (K) colours: C = (1-R'-K) / (1-K); the magenta colour (M) was calculated from the green (G') and black (K) colours: M = (1-G'-K) / (1-K); the yellow colour (Y) was calculated from the blue (B') and black (K) colours: Y = (1-B'-K) / (1-K).

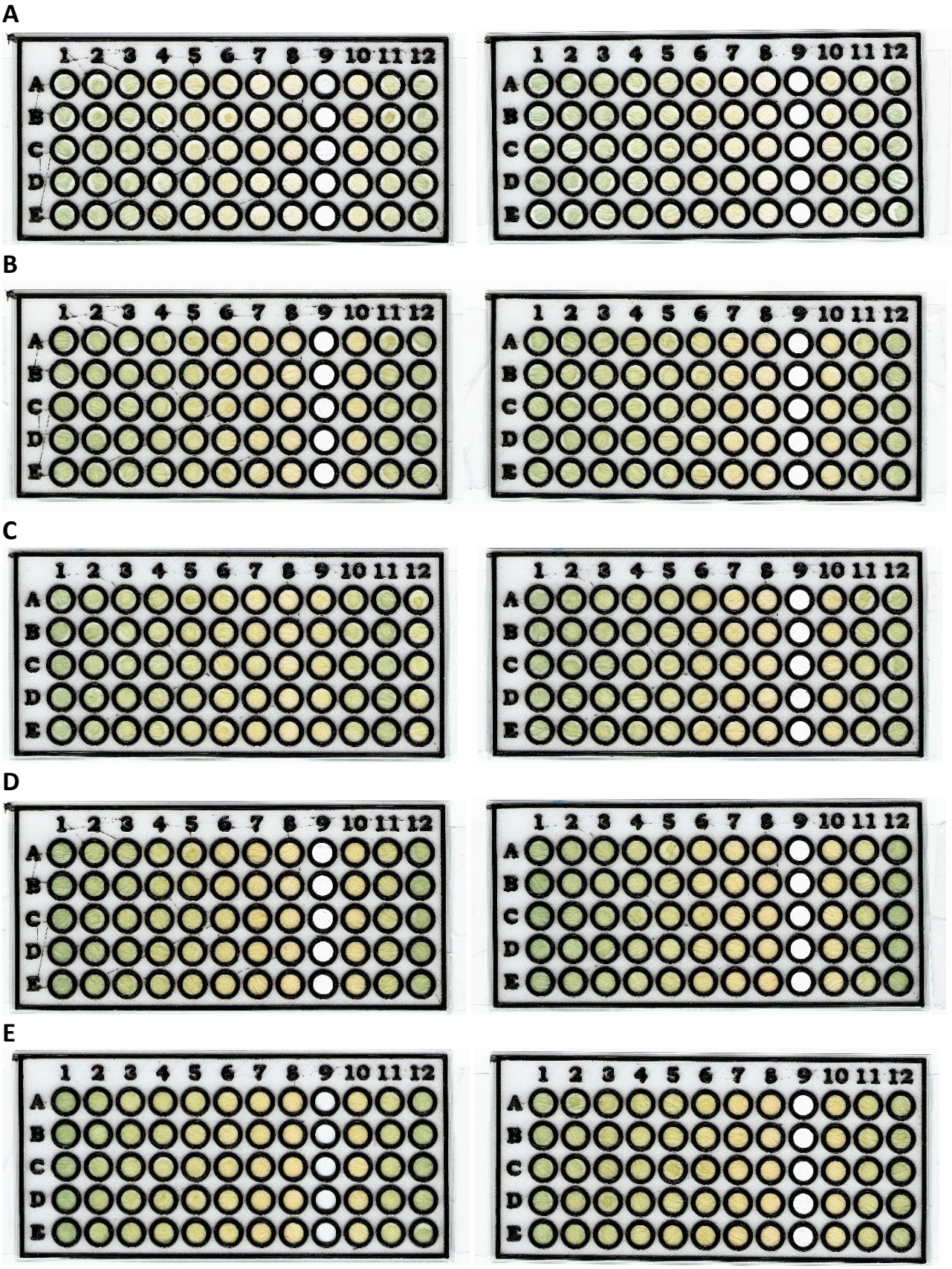

Fig. S2. Image acquisition of the BPB colorimetric assay performed in the PAD at increasing loading volume: 10 µL (A), 12.5 µL (B), 15 µL (C), 20 µL (D), 25 µL (E). Columns 1-8 represent the calibration curve of BSA (2,

1.5, 1.25, 1, 0.75, 0.5, 0.25, 0 mg mL^{-1}); columns 10-12 represent a challenge of 3 concentrations of BSA (0.35, 1, 1.75 mg mL^{-1}). The assays were performed by loading the appropriate volume of BPB reagent + BSA (10:1). For volumes of 10, 12.5 and 15 µL, a waiting time of 10 minutes at room temperature was maintained before image acquisition. For volumes of 20 and 25 µL the PAD was heated at 50°C for 7 minutes before image acquisition. Images were taken by a flatbed scanner.

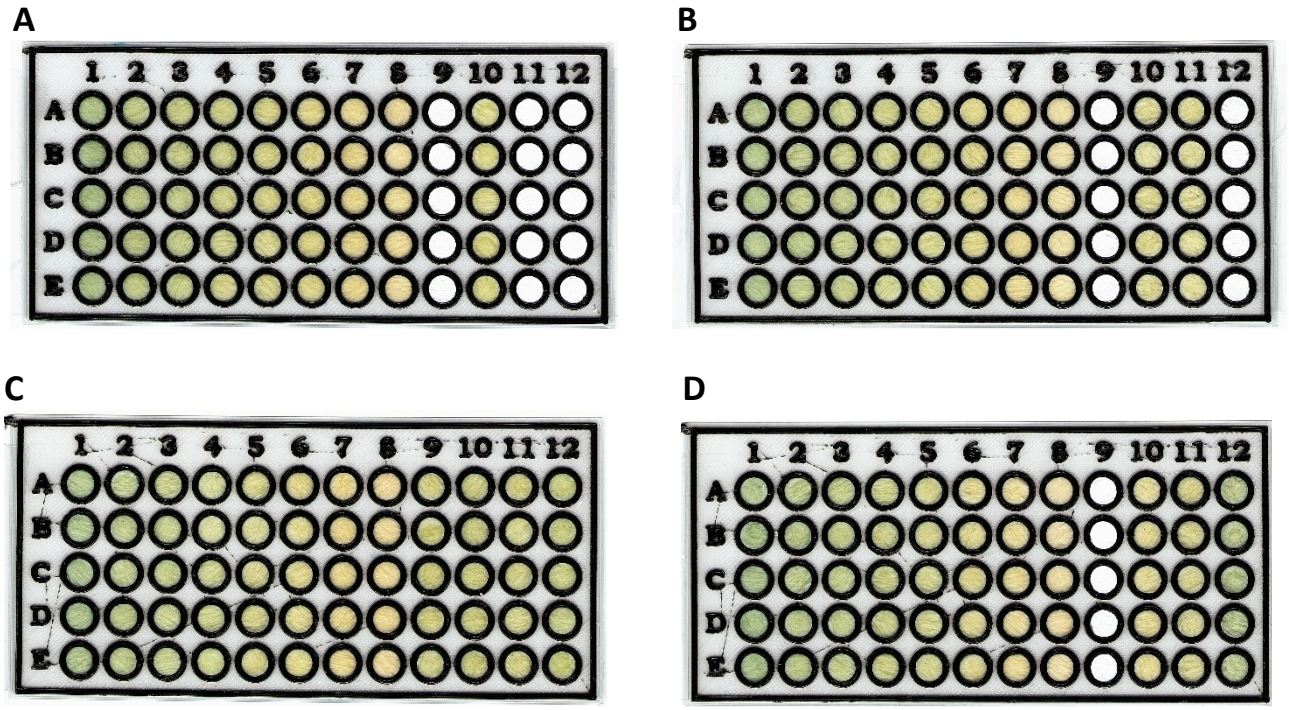

Fig. S3. Image acquisition of the BPB colorimetric assay performed in the PAD for the quantification of protein content in Georgian bee venom (A column 10), soya milk and cow's milk (B columns 10 and 11), Whey P+, Whey P, Eggs P and Vital P protein supplements (C columns 9-12) and in increasing dilutions (1:6, 1:4, 1:2) of mouse brain tissue (D columns 10-12). Columns 1-8 in each panel represent the calibration curve of BSA (2, 1.5, 1.25, 1, 0.75, 0.5, 0.25, 0 mg mL^{-1}). The assay was performed by loading 20 µL of BPB reagent + BSA (10:1) and allowing the PAD to dry for 7 minutes at 50°C. Images were taken by a flatbed scanner.