A Dual Sensor for Biogenic Amines and Oxygen Based on Genipin Immobilized in Edible Calcium Alginate Gel Beads

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Submitted date: 06/05/2020 • Posted date: 07/05/2020

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Citation information: Mallov, Ian; Jeeva, Fiona; Caputo, Chris (2020): A Dual Sensor for Biogenic Amines and Oxygen Based on Genipin Immobilized in Edible Calcium Alginate Gel Beads. ChemRxiv. Preprint.

<https://doi.org/10.26434/chemrxiv.12252323.v1>

Food is often wasted due to real or perceived concerns about preservation and shelf life. Thus, precise, accurate and consumer-friendly methods of indicating whether food is safe for consumers are drawing great interest. The colorimetric sensing of biogenic amines released as food degrades is a potential way of determining the quality of the food. Herein, we report the use of genipin, a naturally occurring iridoid, as a dual colorimetric sensor for both oxygen and biogenic amines. Immobilization of genipin in edible calcium alginate beads demonstrates that it is a capable sensor for amine vapors and can be immobilized in a non-toxic, food-friendly matrix.

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A Dual Sensor for Biogenic Amines and Oxygen Based on Genipin Immobilized in Edible Calcium Alginate Gel Beads

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ABSTRACT: Food is often wasted due to real or perceived concerns about preservation and shelf life. Thus, precise, accurate and consumer-friendly methods of indicating whether food is safe for consumers are drawing great interest. The colorimetric sensing of biogenic amines released as food degrades is a potential way of determining the quality of the food. Herein, we report the use of genipin, a naturally occurring iridoid, as a dual colorimetric sensor for both oxygen and biogenic amines. Immobilization of genipin in edible calcium alginate beads demonstrates that it is a capable sensor for amine vapors and can be immobilized in a non-toxic, food-friendly matrix.

Introduction

Biogenic amines (BAs) are a major indicator of food spoilage, particularly in meat and fish, but also in such food and drink as beer, red wine, mushrooms, and aged cheese.¹ BAs are produced when food is exposed to oxygen gas, allowing for microbial oxidative hydrodecarboxylation of amino acids. They are responsible for the distinctive odor of rotting meat, although adverse effects to human health can occur in concentrations lower than the odor threshold.²⁻⁴ Globally, nearly 1/3 of all food is wasted, thus precise, accurate and consumer-friendly methods of indicating whether food is safe to consumers are drawing great interest.⁵

A number of instrumental methods and techniques to detect BA's have been developed which utilize conventional chromatography methods,² chemoresistivity,^{4,6} or biological sensing.⁷ However, these methods have limitations for practical use in detecting food spoilage, as the analyses require large or specialized equipment, and these impede their application by the public. Progress has been made on this front and an accessible electrical sensor was recently reported utilizing printed electronic gas sensors onto cellulose fibers to detect ammonia.⁸ On the other hand, visual detection sensors have garnered attention as reliable systems which do not require significant instrumentation. Visual sensors can be subdivided into fluorescent or colorimetric sensors. Both benefit from enhanced sensitivity, ease of use and real-time analysis. However, colorimetric sensors have the advantage of being detectable by the naked eye. Considerable research has been undertaken to develop colorimetric sensors for BAs. Some recent highlights of BA detection include copper-containing complexes which have shown selective detection for spermidine and spermine,⁹ light-activated diarylethene probes,¹⁰ aza-bodipy derivatives,¹¹ and derivatives of Meldrum's activated furan^{12,13} (Scheme 1).

Tang, 2016

Hawker and Read De Alaniz, 2017

This Work

Scheme 1 – Recent Examples of Small Molecules for BA Sensing

Previously reported colorimetric BA sensors still have several drawbacks. Many are non-specific for amine substitution (BAs are generally primary amines) or are made of materials which would be incompatible with food (i.e. toxic or non-edible materials). Recent studies have addressed the latter drawback by utilizing the pH sensitivity of anthocyanins found in cabbage extracts to detect BA.^{14,15} However, these sensors are not specific for primary BA. Most significantly, current colorimetric sensors cannot detect the cause of food spoilage, oxygen gas, so false positives could occur through simple detection of amino acids found in the food. To address this issue, modified atmospheric packaging is gaining in popularity as a way of excluding oxygen from food and extending shelf lives.¹⁶

An ideal colorimetric sensor for real-time food spoilage would sense gaseous BAs at low concentrations and rapidly undergo a distinct color change at a reasonable reaction rate. Further, this sensor must have the ability to selectively detect the primary amines as well as the source of the degradation, oxygen gas. This ideal sensor would be composed of cheap, low-toxicity materials derived from edible sources and possess a minimal environmental footprint. We aimed to design a sensor satisfying as many of these criteria as possible.

Genipin, (Scheme 1, bottom) a naturally occurring, colorless iridoid, has found numerous applications in diverse fields, most commonly used as a non-toxic cross-linking reagent for biomaterials.^{17,18} As an edible fruit extract, genipin also has relatively low toxicity; a recent study shows that neither genipin nor the resultant gardenia blue dye exhibit genotoxicity.¹⁹ This transformation from a colorless iridoid to dye can be attributed to the fact that genipin reacts with amines through a double condensation reaction forming an oxidatively unstable

dihydropyridine intermediate (Scheme 1, bottom). In the presence of oxygen, this intermediate further undergoes oxidative oligomerization/polymerization resulting in a brilliant blue dye.²⁰ However, to the best of our knowledge genipin has yet to find applications as a sensor, despite this unique reactivity positioning it as an ideal candidate for a colorimetric sensor for food degradation.

We sought to study the ability of genipin to react with BAs and confirm the selectivity towards a broad range of amines. Herein, we describe the use of genipin as the first dual colorimetric sensor for both O₂ and BAs in solution and provide a proof of concept in an immobilized form.

Experimental Section

Materials. All chemical reagents or solvents were purchased from Fisher Scientific or Millipore Sigma and used as received unless otherwise indicated. Genipin was kindly donated by Inkbox Ink and purity verified independently by ¹H NMR analysis. Food-grade sodium alginate was purchased from BYOB Cocktail Emporium in Toronto and used without purification. NMR spectra were obtained at 25 °C on either a Bruker 700 MHz Spectrometer, Bruker DRX 600 MHz Spectrometer, Bruker ARX 400 MHz Spectrometer or, Bruker ARX 300 MHz Spectrometer. Chemical shifts are reported relative to SiMe₄ and referenced to the residual solvent signal (¹H, ¹³C{¹H}). NMR spectra were analyzed using either TopSpin 4.0.1 or MestReNova 6.0.2-5475 software. Chemical shifts are reported in ppm and coupling constants as scalar values in Hz. The conventional abbreviations were used as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet), br (broad). Absorption measurements were recorded with a Cary 5000 UV-vis-NIR Spectrophotometer from Agilent Technologies. Recordings were obtained at 25 °C and taken with the instrument operating in dual beam mode and referenced to methanol. All absorption experiments were conducted in quartz cuvettes (1cm x 1cm) equipped with a Teflon seal.

UV-visible absorbance detection of biogenic amines in solution 22.6 mg (0.10 mmol) of genipin was dissolved in 45.0 mL methanol in a single-neck round-bottom flask by stirring for five minutes with a magnetic stirrer. For 1:1 solutions of genipin:BA, 0.10 mmol of BA were added to a separate pre-weighed vial and rinsed into the genipin solution with a further 5.0 mL methanol to prepare 50.0 mL of solution of concentrations 2.0 x 10⁻³ M in genipin and BA. For solutions lower in concentration of BA, stock solutions 0.020 M in BA were prepared in methanol. Appropriate volumes of additional methanol were added as needed to dilute to desired concentrations, with total volumes made up to 50.0 mL. Round-bottomed flasks containing solutions were left open to allow exposure to oxygen and stirred for 48-72 hours. Measurement of UV-visible absorbances were carried out by decanting solutions into a graduated cylinder at time of measurement, making up the total volume to 50.0 mL with methanol to compensate for evaporation, decanting back into the round-bottom flask and stirring for 5 minutes to achieve homogeneity. Samples were then transferred into a quartz cuvette for analysis. Single-neck (1" diameter) round-bottom flasks were chosen as reaction vessels after verifying that rates of evaporation of stirred

methanol at room temperature ensured that solution volumes never fell below 40 mL during the course of the reaction.

Preparation of genipin-embedded calcium alginate gel beads. 1, 10, 50, or 100 mg of genipin was dissolved in 10.0 mL of distilled water in a 100 mL beaker. In the case of the addition of 100 mg of genipin, heating to 80 °C was required for full dissolution. 200 mg of sodium alginate powder was added with vigorous stirring to form the sodium alginate gel. Using a variation of the simple dripping (extrusion) technique described by Bhandari et. al.,²¹ a 1 mL disposable syringe without a needle was employed to uptake 1.00 mL of sodium alginate gel. Due to dripping of gel from the outside of the syringe, the first 1-2 drops were dispensed back into the gel mixture. The remainder was dispensed dropwise from a height of 10 cm above the surface of 25.0 mL of a 1.0 %w/v CaCl_2 solution contained in a 125 mL Erlenmeyer flask. 12-15 beads were dispensed in this manner until the syringe contained >0.1 mL of gel, at which point the syringe was re-filled to avoid size decrease of gel beads due to air bubbles inside the syringe, and lack of material. After 100-110 beads had been dispensed in this manner, using 75-80% of the sodium alginate gel mass, the remaining CaCl_2 solution was decanted from the beads and the beads were washed with 10 mL of an aqueous solution of 0.1 M $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ pH 4 buffer. Beads were then dispensed onto a watch glass, separated using tweezers and permitted to stand 1 hour to allow excess water to run onto the glass. The beads were then removed from the watch glass and stored in a sealed vial in a refrigerator at 4 °C.

General Procedure for reaction of genipin immobilized in calcium alginate gel beads with primary amine vapors. 1-10 genipin-embedded calcium alginate gel beads were placed in a 20 mL vial using a clean pair of tweezers. 0.10 mmol of primary amine was added to a 1-dram vial (or Eppendorf tube) which was placed, uncapped, inside the 4-dram vial containing the beads, and the larger vial was sealed with a cap and either left at room temperature or placed in a refrigerator at 4 °C and monitored visually for up to 48 hours days.

In the case where ten gel beads were exposed to 0.10 mmol amine, after 24 hours at room temperature the gel beads remained colourless, while after 48 hours all ten beads had turned blue. In the case where only one bead was used instead of ten beads, the single bead turned deep blue within 24 hours.

To achieve dissolution of gel beads to obtain UV-vis spectra after specified reaction times of 6-48 hours, 10 dyed genipin-containing gel beads were added to 3.5 mL of a 55 mM solution of sodium citrate to allow dissolution of gel beads by cation exchange mediated by the citrate cation chelator.²² Although some of the blue dye became insoluble particulate in this aqueous solution, precluding qualitative UV-vis absorption measurements, enough remained in solution to qualitatively observe the blue dye by UV-vis spectroscopy.

Reaction of genipin immobilized in calcium alginate gel beads with raw chicken. 10 genipin-embedded calcium alginate gel beads produced no more than seven days previously were placed in each of two 20 mL vials using a clean pair of tweezers. A piece of chicken breast purchased

within the previous 24 hours from a commercial grocery store was cut into slices of mass 5-6 grams and one slice was placed in each vial using a separate, clean pair of tweezers, such that the gel beads were separated from the surface of the raw chicken. The vials were sealed with a plastic screw cap. One was left at room temperature, while one was refrigerated at 4 °C. Both samples were monitored visually and photographed at hours 1, 2, 3 and 6.

Results and Discussion

To confirm the selectivity of genipin for primary amines, the reactions with a series of amines in methanol solutions were monitored over periods of 48-72 hours at room temperature. These reactions were performed at concentrations of 2.0×10^{-3} M for both genipin and amine, in open vessels to permit diffusion of O_2 into the system to promote blue dye formation. Benzylamine, *N*-methylbenzylamine, and *N,N*-dimethylbenzylamine were initially tested to represent a series of simple, analogous primary, secondary and tertiary amines, and reactions were monitored by UV-vis absorbance spectroscopy. Consistent with reported literature, formation of blue dye was only observed in the reaction of genipin with the primary benzylamine, with strong visible absorbance exhibited between 550-650 nm (Figure S4). Though solutions of tertiary and secondary amines, *N,N*-dimethylbenzylamine and *N*-methylbenzylamine, did exhibit a colour change to reddish golden, they did not form the blue dye even after extended reaction times. Monitoring the reactions of *N,N*-dimethylbenzylamine and *N*-methylbenzylamine, by 1H NMR spectroscopy with genipin at greater concentrations does indicate a reaction between genipin and amine but it produced an intractable mixture of products. These studies unequivocally show the selectivity of genipin to react with primary amines to form a blue product.

Reactions with biogenic amines putrescine, cadaverine, tyramine and histamine also exhibited formation of deep blue dye (Figure 1). In these cases, an incubation period of 12-24 hours was required before strong absorbances with maxima at 590-610 nm were observed. The incubation time is attributable to the time required for growth of conjugated oligomers absorbing in the red region. Although reactions were monitored for up to 120 hours, in all cases, λ_{max} and molar attenuation coefficient values demonstrated only minimal change after 48 hours (*e.g.* Figure S5). Interestingly, molar attenuation coefficients varied widely between reactions of genipin with different BA, ranging from 1.2×10^2 Lmol $^{-1}$ cm $^{-1}$ in putrescine to 4.4×10^3 Lmol $^{-1}$ cm $^{-1}$ in tyramine. It is not obvious from differences in molecular structure of the amines why significant variance is observed in molar attenuation coefficients of the solutions of dyes.

Figure 1 – Stacked UV-vis Spectra for the reaction of genipin with BAs. Dilution factors – 1,4-diaminobutane (no dilution); 1,5-diaminopentane (5x dilution); Tyramine (10x dilution); Histamine (20x dilution).

Unsurprisingly, when reactions were carried out at increased concentrations of genipin and amine, the blue dye formed much more rapidly, for example within 4 hours at concentrations of 0.20 M (Figure S10)).

From these solution studies, the limit of visual detection of amines with a 0.50 M solution of genipin in 50:50 methanol:water was found to vary depending on BA used. Putrescine and tyramine, the tested amines whose dyes exhibited the lowest and highest molar attenuation coefficients, respectively, were examined. Solutions of genipin in methanol exhibit a dull golden tinge, while presence of the blue dyes were confirmed visually by a change in colour to dull green, the result of the presence of both blue dye and the golden hue from remaining unreacted genipin. Tyramine could be visually detected at concentrations of 6.9 ppm after 24 hours (Figure S27), while the detection limit for putrescine was nearly an order of magnitude higher at 44 ppm after 24 hours. This trend correlates with the measured trend in molar attenuation coefficient measured for the dyes incorporating putrescine or tyramine units (converted from molarity).

Enticingly, the reaction of putrescine with genipin in the absence of oxygen produced a different response (Figure 2). Using degassed water and an atmosphere of nitrogen, the solutions produced a golden red coloration with no indication of the blue response as indicated by the λ_{max} at ~ 600 nm. Upon exposure to air after 48 hours, the solutions rapidly turned blue. To explore the sensitivity of the genipin and amine intermediate to oxygen gas, we found even 0.5% (5000 ppm) of oxygen gas (converted from moles of H_2O_2 , see SI) added under an atmosphere of N_2 were enough to trigger the oxidative oligomerization reaction turning the sample blue over a

period of 24 hours. This sensitivity is below the oxygen threshold established in modified atmospheric packaging ($\sim 2\%$) and in the desired range of sensitivity ($0.5\text{-}2\% \text{ O}_2$ level).¹⁶

Figure 2 - UV-vis absorbance spectra of 2.0 mM solutions of genipin in methanol in reaction with 2.0mM of 1,4-diaminobutane in the presence (blue) and absence (red) of O_2 after 48 hours.

To demonstrate the applicability of our sensing platform for food spoilage detection, an immobilization method for genipin was developed using a calcium alginate gel matrix. Alginate gels are common edible food thickening agents derived from alginic acid sourced from seaweed or brown algae.²² Calcium alginate gel beads are easily produced from sodium alginate and permit penetration of small molecules through their pores.²¹ Genipin was embedded in these beads through modifying a literature procedure for the impregnation of calcium alginate gel beads with enzymes.²¹ After extensive screening, it was found that a concentration of 5 mg/mL of genipin in water, approximately equivalent to the room-temperature solubility of genipin in water, resulted in the most responsive beads. As a proof of concept, we selected the most volatile biogenic amine, putrescine, to test the sensing capabilities of the beads. Immobilized genipin-containing beads were exposed to the gaseous BA by adding 0.1 mmol of putrescine to an open 1-dram vial and sealing the 1-dram vial inside a 4-dram vial containing 10 genipin-infused beads. The beads responded as expected and a blue colour developed upon exposure to BA vapors within 24 hours (Figure 3). To mimic conditions for food storage, these experiments were also undertaken at 4 °C, and encouragingly the beads did not exhibit any color change, even after 48 hours (Figure S28). Gel beads could subsequently be dissolved in an aqueous citric acid solution for UV-vis absorbance analysis (Figure 3), although undissolved particulate matter interfered with quantitative measurements.

In analyzing the absorbance of solutions obtained from dissolving gel beads exposed for 48 hours to vials containing varying amounts of putrescine (Figure S29), it is evident that increasing amounts of amine result in increased intensity of the blue dye. The vapor pressure of linear putrescine has been reported as 259.3 Pa at 300.7K.²³ Using the reported vapor pressure at 300.7

K as an approximation for the vapor pressure at room temperature, it can be calculated from the ideal gas law that for the 18 mL closed volume of the 4-dram vial, evaporation of <0.2 mg of putrescine is required to reach equilibrium vapor pressure. Upon dissolution after reaction, solutions of gel beads exposed to 0.3 mg, 1.2 mg and 12 mg of putrescine in open one-dram vials demonstrated significantly increased absorbance as the amount of amine was increased (Figure S29). Thus, it can be hypothesized that the intensity of the absorbance is more correctly correlated to the absolute amount of amine available for reaction, and the identity of that amine, than the vapor pressure.

Figure 3: Genipin-impregnated beads after 24 hours exposure to putrescine vapours at room temperature

Having established the sensor's response to gaseous amines, we sought to test them against real food samples. Samples of chicken breast (5-6 grams each) were freshly prepared and stored in sealed vials with the genipin-impregnated beads. With the beads in close proximity to the meat, the sensors behaved as expected and turned blue upon exposure. Samples were stored both at room temperature and in a refrigerator at 4 °C to mimic typical storage conditions. To quantify the color difference between the beads, ΔE^* values were determined following commission internationale de l'éclairage (CIE) guidelines (Figure 4). A ΔE^* value >5 is considered to be distinguishable by the naked eye.¹² This approach has previously been used to determine the effectiveness of small molecule amine sensors.¹² The sensors were monitored over the course of 6 hours with images taken at time points of 1, 2, 3 and 6 hours (Table S1). ΔE^* values of 38 and 0

were obtained after 6 hours for the samples at room temperature and at 4 °C, respectively. These values correspond to a significant blue coloration of the beads at room temperature versus no visible change in the refrigerator (Figure 4). The rate of colour appearance in these indicator beads is consistent with the USDA health advisory on raw poultry, which warns against leaving raw poultry at room temperature for longer than two hours.²⁴

Figure 4: Change in color of Genipin Impregnated Beads in the presence of chicken, room temperature (blue) and 4 °C (orange).

Conclusion

In summary, this proof-of-principal study indicates that genipin can act as a highly selective dual sensor for both biogenic amines and O_2 . This uniquely positions genipin as an ideal colorimetric sensor for food spoilage as it can detect the root cause of this process through off-gases. Moreover, by immobilizing the sensor in an edible calcium alginate matrix, biogenic amine vapors were detected at very low concentrations. This was extended to monitoring chicken spoilage by the naked eye, indicating its potential in food spoilage detection.

Acknowledgement

The authors gratefully acknowledge financial support from NSERC of Canada, the Canadian Foundation for Innovation and Inkbox Ink. CBC is grateful for the award of a Canada Research Chair. The authors thank Professor Thomas Baumgartner for the use of the UV-vis spectrophotometer and valuable feedback.

References

1. Santos MHS. Biogenic amines: their importance in foods. *Int J Food Microbiol.* 29:213–31 (1996);
2. Önal A. A review: Current analytical methods for the determination of biogenic amines in foods. *Food Chem.* 103:1475–86 (2007);
3. Naila A, Flint S, Fletcher G, Bremer P, Meerdink G. Control of Biogenic Amines in Food-Existing and Emerging Approaches. *J Food Sci.* 75:R139–50 (2010);

4. Liu SF, Petty AR, Sazama GT, Swager TM. Single-Walled Carbon Nanotube/Metalloporphyrin Composites for the Chemiresistive Detection of Amines and Meat Spoilage. *Angew. Chem. Int Ed.* 54:6554–7 (2015);
5. Aschemann-Witzel J. Waste not, want not, emit less: Reducing food waste in the supply chain and at home can help to reduce carbon emissions. Vol. 352, *Science*. 352:408–9 (2016);
6. Sotzing GA, Phend JN, Grubbs RH, Lewis NS. Highly Sensitive Detection and Discrimination of Biogenic Amines Utilizing Arrays of Polyaniline/Carbon Black Composite Vapor Detectors. *Chem Mater*. 12:593–5 (2000);
7. Yousefi H, Ali MM, Su H-M, Filipe CDM, Didar TF. Sentinel Wraps: Real-Time Monitoring of Food Contamination by Printing DNAzyme Probes on Food Packaging. *ACS Nano*. 12:3287–94 (2018);
8. Barandun G, Soprani M, Naficy S, Grell M, Kasimatis M, Chiu KL, Ponzoni A, Güder F. Cellulose Fibers Enable Near-Zero-Cost Electrical Sensing of Water-Soluble Gases. *ACS Sensors*. 4:1662–9 (2019);
9. Chopra S, Singh A, Venugopalan P, Singh N, Kaur N. Organic nanoparticles for visual detection of spermidine and spermine in vapors and aqueous phase. *ACS Sustain Chem Eng*. 5:1287–96 (2017)
10. Valderrey V, Bonasera A, Fredrich S, Hecht S. Light-Activated Sensitive Probes for Amine Detection. *Angew. Chem. Int Ed.* 56:1914–8 (2017);
11. Li L, Li W, Ran X, Wang L, Tang H, Cao D. A highly efficient, colorimetric and fluorescent probe for recognition of aliphatic primary amines based on a unique cascade chromophore reaction. *Chem Commun*. 55:9789–92 (2019);
12. Diaz YJ, Page ZA, Knight AS, Treat NJ, Hemmer JR, Hawker CJ, Read De Alaniz J. A Versatile and Highly Selective Colorimetric Sensor for the Detection of Amines. *Chem. Eur J*. 23:3562–6 (2017);
13. Chen Q, Diaz YJ, Hawker MC, Martinez MR, Page ZA, Xiao-An Zhang S, Hawker CJ, Read de Alaniz J. Stable Activated Furan and Donor-Acceptor Stenhouse Adduct Polymer Conjugates as Chemical and Thermal Sensors. *Macromolecules*. 52:4370–5 (2019);
14. Dudnyk I, Janeček E-R, Vaucher-Joset J, Stellacci F. Edible sensors for meat and seafood freshness. *Sensors Actuators B Chem*. 259:1108–12 (2018);
15. Huang X, Zou X, Shi J, Guo Y, Zhao J, Zhang J, Hao L. Determination of pork spoilage by colorimetric gas sensor array based on natural pigments. *Food Chem*. 145:549–54 (2014);
16. Mills A. Oxygen indicators and intelligent inks for packaging food. *Chem. Soc. Rev*. 34:1003–11 (2005).
17. Yan LP, Wang YJ, Ren L, Wu G, Caridade SG, Fan JB, Wang LY, Ji PH, Oliveira JM, Oliveira JT, Mano JF, Reis RL. Genipin-cross-linked collagen/chitosan biomimetic scaffolds for

- articular cartilage tissue engineering applications. *J Biomed Mater Res Part A*. 95A:465–75 (2010);
18. Butler MF, Ng Y-F, Pudney PDA. Mechanism and kinetics of the crosslinking reaction between biopolymers containing primary amine groups and genipin. *J Polym Sci Part A Polym Chem*. 41:3941–53 (2003);
 19. Tsai C-C, Huang R-N, Sung H-W, Liang HC. In vitro evaluation of the genotoxicity of a naturally occurring crosslinking agent (genipin) for biologic tissue fixation. *J Biomed Mater Res*. 52:58–65 (2000);
 20. Touyama R, Takeda Y, Inoue K, Kawamura Ik, Yatsuzuka M, Ikumoto T, Shingu T, Yokoi T, Inouye H. Studies on the Blue Pigments Produced from Genipin and Methylamine. I. Structures of the Brownish-Red Pigments, Intermediates Leading to the Blue Pigments. *Chem Pharm Bull*. 42:668–73 (1994);
 21. Ching SH, Bansal N, Bhandari B. Alginate gel particles—A review of production techniques and physical properties. *Crit Rev Food Sci Nutr*. 57:1133–52 (2017);
 22. Goh CH, Heng PWS, Chan LW. Alginates as a useful natural polymer for microencapsulation and therapeutic applications. Vol. 88, Carbohydrate Polymers. p. 1–12 2012.
 23. Pozdeev VA, Verevkin SP. Vapor pressure and enthalpy of vaporization of linear aliphatic alkanediamines. *J Chem Thermodyn*. 43:1791–9 (2011);
 24. https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/keep-food-safe-food-safety-basics/ct_index

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Supporting Information

A Dual Sensor for Biogenic Amines and Oxygen Based on Genipin Immobilized in Edible Calcium Alginate Gel Beads

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1. General Considerations

All chemical reagents or solvents were purchased from Fisher Scientific or Millipore Sigma and used as received unless otherwise indicated. Genipin was kindly donated by Inkbox Ink and purity verified independently by ^1H NMR analysis. Food-grade sodium alginate was purchased from BYOB Cocktail Emporium in Toronto and used without purification. NMR spectra were obtained at 25 °C on either a Bruker 700 MHz Spectrometer, Bruker DRX 600 MHz Spectrometer, Bruker ARX 400 MHz Spectrometer or Bruker ARX 300 MHz Spectrometer. Chemical shifts are reported relative to SiMe_4 and referenced to the residual solvent signal (^1H , $^{13}\text{C}\{^1\text{H}\}$). NMR spectra were analyzed using either TopSpin 4.0.1 or MestReNova 6.0.2-5475 software. Chemical shifts are reported in ppm and coupling constants as scalar values in Hz. The conventional abbreviations were used as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet), br (broad). Absorption measurements were recorded with a Cary 5000 UV-Vis-NIR Spectrophotometer from Agilent Technologies. Recordings were obtained at 25 °C and taken with the instrument operating in dual beam mode and referenced to methanol. All absorption experiments were conducted in quartz cuvettes (1cm x 1cm) equipped with a Teflon seal.

2. UV-Visible Absorbance Spectra of Starting Materials

Figure S1 - UV-visible absorbance spectrum of 2.0×10^{-3} M MeOH solution of putrescine.

Figure S2 – UV-visible absorbance spectrum of 2.0×10^{-3} M MeOH solution of genipin

Figure S3 - UV-visible absorbance spectrum of 8.0×10^{-5} M MeOH solution of genipin

3. UV-Visible Absorbance Spectra – Reaction of Amines with Genipin in Solution

General Procedure

Genipin (22.6 mg, 0.10 mmol) was dissolved in 45.0 mL methanol in a single-neck round-bottom flask by stirring for five minutes with a magnetic stirrer. For 1:1 solutions of genipin:amine, 0.10 mmol of the amine of choice was added to a separate pre-weighed vial and rinsed into the genipin solution with a further 5.0 mL methanol to prepare 50.0 mL of solution of concentrations 2.0×10^{-3} M in genipin and amine. For solutions lower in concentration of amine, stock solutions 0.020 M in amine were prepared in methanol, with appropriate volumes diluted with additional methanol as needed and added to the methanolic genipin solution, with total volumes made up to 50.0 mL. Round-bottomed flasks containing solutions were left open to allow exposure to oxygen and stirred for up to 72 hours. Measurement of UV-visible absorbances were carried out by decanting solutions into a graduated cylinder at time of measurement, making up the total volume to 50.0 mL with methanol to compensate for evaporation, decanting back into the round-bottom flask and stirring for 5 minutes to achieve homogeneity. Samples were then transferred into a quartz cuvette for analysis. Single-neck (1" diameter) round-bottom flasks were chosen as reaction vessels after verifying that rates of evaporation of stirred methanol at room temperature ensured that solution volumes never fell below 40 mL. Samples exhibiting peak absorbances >1 in the visible range were diluted by 2X, 5X, or 10X and absorbance spectra measured at these concentrations.

3.1 Reactions of Benzylamines with Genipin

Figure S4 – UV-visible absorbance spectra of 2.0×10^{-3} M solutions of benzylamine, N-methylbenzylamine, or N,N-dimethylbenzylamine solutions after reaction with genipin at $[2.0 \times 10^{-3}$ M] after 48h

3.2 Reactions of Genipin with Putrescine (1,4-Diaminobutane)

Figure S5 - UV-visible absorbance spectra of a MeOH solution 2.0×10^{-3} M in both genipin and putrescine monitored over 1-120 h.

Exclusion of Oxygen from Reaction – The general procedure was slightly modified to utilize a Schlenk flask or glovebox techniques to ensure an N₂ atmosphere throughout the reaction.

Figure S6 - UV-visible absorbance spectra of MeOH solution 2.0×10^{-3} M in both genipin and putrescine after 24h in the presence and absence of O₂

Figure S7 - UV-Visible absorbance spectra of MeOH solution 2.0×10^{-3} M in both genipin and putrescine after 48h in the presence and absence of O₂

Figure S8 - Combined UV-visible absorbance spectra of MeOH solutions 2.0×10^{-3} M in both genipin and putrescine after 24-48h in the presence and absence of O₂

3.3 Reaction of Genipin with Putrescine With Addition of Chemically-Generated Oxygen

Under a nitrogen atmosphere inside a glove box, two portions of genipin (23 mg, 0.10 mmol) were dissolved in 8.0 mL of de-gassed methanol in separate, single-neck 50 mL round-bottom flasks. Putrescine (8.8 mg, 0.10 mmol) was added to two separate, pre-weighed vials and rinsed into the genipin solutions with a further 2.0 mL methanol, to prepare two 10.0 mL solutions of concentrations 1.0×10^{-2} M in genipin and putrescine. A magnetic stir bar was added to both flasks, both were stoppered and allowed to stir for 24 hours.

After 24 hours, both reaction mixtures appeared reddish-golden in colour. Stirring was stopped for both solutions. The stopper was removed from one round bottom flask and a 1-dram vial containing 3-5 mg (0.02-0.03 mmol) KMnO_4 in 0.5 mL distilled H_2O was placed inside such that the mouth of the vial rested well above the level of the genipin-amine solution. A septum was placed over the mouth of the round bottom flask. 10 μL of a 3% aqueous H_2O_2 solution (9 μmol H_2O_2) was added to the KMnO_4 solution via syringe to evolve 9 μmol O_2 gas (0.2 mL, equating to 0.5% headspace volume, 5000 ppm) according to the balanced reaction **S1** and calculation **S2**:

$$\text{Equation S2: } V_{\text{O}_2} = nRT/P = (9 \times 10^{-6} \text{ mol})(0.08206 \text{ L atm/mol K})(298 \text{ K}) / 1.0 \text{ atm} = 2.0 \times 10^{-4} \text{ L}$$

The O₂ generated by this reaction was allowed to diffuse into the headspace of the flask for 24 hours. After about 1 hour, the solution with the oxygen added to the headspace appeared slightly darker. After 24 hours, the solution with the O₂ added to the headspace appeared the deep blue typical of genipin reaction with amines.

Measurement of UV-visible absorbances were carried out by decanting solutions into a graduated cylinder, making up the total volume to 10.0 mL with methanol to compensate for evaporation, decanting back into the round-bottom flask and stirring for 5 minutes to achieve homogeneity. Samples were removed from each solution, diluted by a factor of 5X, decanted into sealable quartz cuvettes to prevent additional O₂ exposure prior to measurement, and sealed. Cuvettes were then removed from the glove box and UV-visible absorption spectra obtained (Figure S9).

Figure S9 - UV-visible absorbance spectra of MeOH solutions 1.0×10^{-2} M in both genipin and putrescine after reaction under N_2 atmosphere, with the spectrum in red representing absorbance of the solution with no added O_2 and the spectrum in blue after 24h in the presence of 5000 ppm O_2 added by reaction of H_2O_2 and $KMnO_4$ solutions.

Figure S10 - UV-visible absorbance spectra of MeOH reaction mixtures 0.20 M in both genipin and putrescine in MeOH after 4h 7h reaction times (diluted at time of measurement to 2.0×10^{-3} M).

Figure S11 - UV-visible absorbance spectra of reaction mixtures 2.0×10^{-3} M in both genipin and putrescine in MeOH after 48h. Samples run at varying concentrations

Figure S12 - UV-visible absorbances at $\lambda_{\max} = 605 \text{ nm}$ for varying dilutions from a stock solution of concentration $2.0 \times 10^{-3} \text{ M}$ in both genipin and putrescine in MeOH after 48 h

Molar Absorptivity:

$$\epsilon = A/c \cdot l = 1.00 \text{ cm}; A/c = 124.14 \text{ L/mol thus } \epsilon = 1.2 \times 10^2 \text{ L/mol cm}$$

Figure S13 - Comparison of UV-visible absorbance spectra of reaction mixtures 2.0×10^{-3} M in both genipin and putrescine and 2.0×10^{-1} M (this sample diluted 100x) in MeOH After 48h.

3.4 Reactions of Genipin with Cadaverine (1,5-Diaminopentane)

Figure S14 - UV-visible absorbance spectra of MeOH solutions 2.0×10^{-3} M in both cadaverine and genipin over 1-48h

Figure S15 - UV-visible absorbance spectra of MeOH solutions $2.0 \times 10^{-3}\text{M}$ in both cadaverine and genipin over diluted 5X 1-48h

Figure S16 - UV-visible absorbance spectra of MeOH solutions $2.0 \times 10^{-3}\text{M}$ in both cadaverine and genipin after 48h diluted by various factors.

Figure S17 Plot of absorbance versus molar concentration of genipin/cadaverine solutions at 48h.

Molar Absorptivity:

$\epsilon = A/cl$ $l = 1.00$ cm; $A/c = 1822.9$ L/mol thus $\epsilon = 1.8 \times 10^3$ L/mol cm

3.4 Reactions of Genipin with Tyramine

Figure S18 - UV-visible absorbance spectra of MeOH solutions 2.0×10^{-3} M in both genipin and tyramine 2.0×10^{-3} M over 1-48h

Figure S19 - UV-visible absorbance spectra of MeOH solutions 2.0×10^{-3} M in both genipin and tyramine 2.0×10^{-3} M over 1-48h Samples diluted 10x at time of measurement.

Figure S20 - UV-visible absorbance spectra of MeOH solutions 2.0×10^{-3} M in both genipin and tyramine 2.0×10^{-3} M over 1-48h at various dilutions

Figure S21 Plot of absorbance versus molar concentration of genipin/tyramine solutions at 48h

Molar Absorptivity:

$\epsilon = A/cl$ $l = 1.00$ cm; $A/c = 4378.4$ L/mol thus $\epsilon = 4.4 \times 10^3$ L/mol cm

3.5 Reactions of Genipin with Histamine

Figure S22 - UV-visible absorbance spectra of MeOH solutions 2.0×10^{-3} M in both histamine and genipin over 1-48h.

Figure S23 - UV-visible absorbance spectra of MeOH solution 2.0×10^{-3} M in both histamine and genipin after 48h and diluted 2X at time of measurement.

Figure S24 - UV-visible absorbance spectra of MeOH solutions 2.0×10^{-3} M in both genipin and histamine 2.0×10^{-3} M over 1-48h at various dilutions

Figure S25 Plot of absorbance versus molar concentration of genipin/histamine solutions at 48 h

Molar Absorptivity:

$\epsilon = A/cl$ $l = 1.00$ cm; $A/c = 593.97$ L/mol thus $\epsilon = 6.0 \times 10^2$ L/mol cm

3.6 Estimating the Visual Detection Limit of Amines with a 0.500 M Solution of Genipin

20.0 mL of 0.500 M methanolic solution of genipin was prepared by dissolving 2.26 g (0.0100 mmol) genipin in a 50:50 mixture of 10.0 mL methanol and 10.0 mL water in a 50 mL round bottom flask (50:50 water:methanol demonstrated experimentally greater genipin solubility than 100% methanol or 100% water)

Of the UV-visible absorbance spectra of 1:1 solutions of genipin with biogenic amines putrescine, cadaverine, tyramine, and histamine, the blue dye formed by the reaction of genipin with tyramine exhibited the largest molar absorptivity, at 4.4×10^3 L/mol cm. Thus, visual detection of tyramine should be observable at the lowest possible concentration.

A 0.100 M solution of tyramine in methanol was prepared by dissolving 137 mg (1.00 mmol) tyramine in 10.0 mL methanol. A stock solution with a concentration of 1.0×10^{-3} M was prepared by diluting 20 μ L of the 0.100 M solution to a total volume of 2.0 mL with methanol.

35 μ L of the stock solution of tyramine concentration 1.0×10^{-3} M was then added to 3.5 mL of the 0.500 M solution of genipin to produce a solution 1.0×10^{-5} M (1.4 ppm) in tyramine (Solution 1).

165 μ L of the stock solution of tyramine concentration 1.0×10^{-3} M was then added to 3.3 mL of the 0.500 M solution of genipin to produce a solution 5.0×10^{-5} M (6.9 ppm) in tyramine (Solution 2).

Solutions were sealed in 1-dram vials to minimize headspace for evaporation and left for 48 hours. After 48 hours, Solution 1 appeared identical to the naked eye to a solution containing only 0.500 M genipin, while Solution 2 had taken on a greenish colour (Figure S27).

UV-visible absorbance spectrum was obtained for Solution 2 (Figure S26).

Figure S26 -UV-Visible absorbance spectrum of reaction mixture of tyramine at 5.0×10^{-5} M concentration and genipin at 0.50 M concentration in 50:50 MeOH:H₂O After 48h.

Figure S27: 0.50M solutions of genipin in 50:50 water/methanol spiked with 1.4 ppm tyramine (left) and 6.9 ppm tyramine (right)

4. Colorimetric Response of Genipin-Infused Gel Beads Exposed to Amine Vapor

Preparation of Genipin-Impregnated Calcium Alginate Gel Beads

1, 10, 50, or 100 mg of genipin was dissolved in 10.0 mL of distilled water in a 100 mL beaker. In the case of the addition of 100 mg of genipin, heating to 80 °C was required for full dissolution. 200 mg of sodium alginate powder was added with vigorous stirring to form the sodium alginate gel. Using a variation of the simple dripping (extrusion) technique described in the review by Bhandari et. al. (see reference 20 in the main manuscript) a 1 mL disposable syringe without a needle was employed to uptake 1.00 mL of sodium alginate gel. Due to dripping of gel from the outside of the syringe, the first 1-2 drops were dispensed back into the gel mixture. The remainder was dispensed dropwise from a height of 10 cm above the surface of 25.0 mL of a 1.0 %w/v CaCl_2 solution contained in a 125 mL Erlenmeyer flask. 12-15 beads were dispensed in this manner until the syringe contained >0.1 mL of gel, at which point the syringe was re-filled to avoid size decrease of gel beads due to air bubbles inside the syringe and lack of material. After 100-110 beads had been dispensed in this manner, using 75-80% of the sodium alginate gel mass, the remaining CaCl_2 solution was decanted from the beads and the beads were washed with 10 mL of an aqueous solution of 0.1 M $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ buffer. Beads were then dispensed onto a watch glass, separated using tweezers and let stand 1 hour to allow excess water to run onto the glass. The beads were then removed from the watch glass and stored in a sealed vial in a refrigerator at 4 °C.

4.1 Genipin-Infused Gel Beads Exposed to Putrescine Vapor

General Procedure

1-10 genipin-embedded calcium alginate gel beads were placed in a 20 mL vial using a clean pair of tweezers. 0.10 mmol of primary amine was added to a 1 dram vial (or Eppendorf tube) which was placed, uncapped, inside the 20 mL vial containing the beads, and the larger vial was sealed with a cap and either left at room temperature or placed in a refrigerator at 4 °C and monitored visually for up to 48 hours.

In the case where 10 gel beads were exposed to 0.10 mmol amine, after 24 hours at room temperature the gel beads remained colourless, while after 48 hours all ten beads had turned blue. In the case where only one bead was used instead of ten beads, the single bead turned deep blue within twenty-four hours.

Dissolution of gel beads to obtain UV spectra after specified reaction times of 6-48 hours, 10 dyed genipin-containing gel beads were dissolved in 3.5 mL of a 55 mM solution of sodium citrate to allow dissolution of gel beads by to cation exchange mediated by the citrate cation chelator.²¹

Figure S28: A) gel beads at $t = 0$ with putrescine beaker **B)** gel beads after 24h reaction with putrescine vapour **C)** comparison of gel beads in reaction with putrescine vapour after 48h at room temperature (left vial) and in the refrigerator (right vial)

Figure S29: UV-visible absorbance spectra of dissolved genipin-infused gel beads after exposure to 0.3 mg, 1.2 mg, or 12 mg of putrescine.

Figure S30: UV-visible absorbance spectra of dissolved gel beads containing 4 different concentrations of genipin, after exposure to 0.10 mmol (8.8 mg) putrescine for 48 h

5. Colorimetric Response of Genipin-Infused Gel Beads Exposed to Raw Chicken

Table S1 - Genipin-infused calcium alginate gel beads exposed to freshly purchased raw chicken at room temperature (left) and refrigerated at 4 °C (right)

Time	Room Temperature	Refrigerator (4 °C)
1 hour
2 hours

Quantification of color from images

The software Adobe Photoshop was used to quantify colors in images using International Commission on Illumination (CIE) guidelines with chromatic values $L^*a^*b^*$ representing lightness (L^*) and color opponent red/green (a^*) and yellow/blue (b^*) dimensions. We followed the procedure outlined by Read de Alaniz¹ where the Delta E (ΔE^*) value (the distance between two chromatic values in color space) is used as a metric to differentiate two colors.¹

¹ Chem. Eur. J. **2017**, 23, 3562-3566; J. Food Eng. **2004**, 61, 137-142; Measuring Color: Fourth Edition, **2011**; Food Res. Int. **2006**, 39, 1092-1098.

Figure S31: Change in color as measured by ΔE^* of the beads at room temperature relative to the beads in the fridge.

6. NMR Spectroscopy

General Procedure for Reaction of Genipin with Benzylamine, N-methylbenzylamine, and N,N-dimethylbenzylamine

To a solution of 23 mg (0.10 mmol) genipin in 1 mL CD₃OD in a 4 dram vial was added a solution of 0.10 mmol of the respective amine. A small stir bar was added and solutions were allowed to stir for 24h. An aliquot of the resulting blue solution was then decanted into an NMR tube and ¹H NMR spectrum was obtained.

¹H NMR spectrum of genipin in CD₃OD

^1H NMR spectrum of benzylamine in CD_3OD

^1H NMR spectrum of reaction mixture of benzylamine and genipin in CD_3OD

^1H NMR spectrum of N-methylbenzylamine in CD_3OD

^1H NMR spectrum of reaction mixture of N-methylbenzylamine and genipin in CD_3OD

^1H NMR spectrum of N,N-dimethylbenzylamine in CD_3OD

^1H NMR spectrum of reaction mixture of N,N-dimethylbenzylamine and genipin in CD_3OD

Amine Sensor SI.pdf (2.18 MiB)

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A Dual Sensor for Biogenic Amines and Oxygen Based on Genipin Immobilized in Edible Calcium Alginate Gel Beads

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ABSTRACT: Food is often wasted due to real or perceived concerns about preservation and shelf life. Thus, precise, accurate and consumer-friendly methods of indicating whether food is safe for consumers are drawing great interest. The colorimetric sensing of biogenic amines released as food degrades is a potential way of determining the quality of the food. Herein, we report the use of genipin, a naturally occurring iridoid, as a dual colorimetric sensor for both oxygen and biogenic amines. Immobilization of genipin in edible calcium alginate beads demonstrates that it is a capable sensor for amine vapors and can be immobilized in a non-toxic, food-friendly matrix.

Introduction

Biogenic amines (BAs) are a major indicator of food spoilage, particularly in meat and fish, but also in such food and drink as beer, red wine, mushrooms, and aged cheese.¹ BAs are produced when food is exposed to oxygen gas, allowing for microbial oxidative hydrodecarboxylation of amino acids. They are responsible for the distinctive odor of rotting meat, although adverse effects to human health can occur in concentrations lower than the odor threshold.²⁻⁴ Globally, nearly 1/3 of all food is wasted, thus precise, accurate and consumer-friendly methods of indicating whether food is safe to consumers are drawing great interest.⁵

A number of instrumental methods and techniques to detect BA's have been developed which utilize conventional chromatography methods,² chemoresistivity,^{4,6} or biological sensing.⁷ However, these methods have limitations for practical use in detecting food spoilage, as the analyses require large or specialized equipment, and these impede their application by the public. Progress has been made on this front and an accessible electrical sensor was recently reported utilizing printed electronic gas sensors onto cellulose fibers to detect ammonia.⁸ On the other hand, visual detection sensors have garnered attention as reliable systems which do not require significant instrumentation. Visual sensors can be subdivided into fluorescent or colorimetric sensors. Both benefit from enhanced sensitivity, ease of use and real-time analysis. However, colorimetric sensors have the advantage of being detectable by the naked eye. Considerable research has been undertaken to develop colorimetric sensors for BAs. Some recent highlights of BA detection include copper-containing complexes which have shown selective detection for spermidine and spermine,⁹ light-activated diarylethene probes,¹⁰ azabodipy derivatives,¹¹ and derivatives of Meldrum's activated furan^{12,13} (Scheme 1).

Scheme 1 – Recent Examples of Small Molecules for BA Sensing

Previously reported colorimetric BA sensors still have several drawbacks. Many are non-specific for amine substitution (BAs are generally primary amines) or are made of materials which would be incompatible with food (i.e. toxic or non-edible materials). Recent studies have addressed the latter drawback by utilizing the pH sensitivity of anthocyanins found in cabbage extracts to detect BA.^{14,15} However, these sensors are not specific for primary BA. Most significantly, current colorimetric sensors cannot detect the cause of food spoilage, oxygen gas, so false positives could occur through simple detection of amino acids found in the food. To address this issue, modified atmospheric packaging is gaining in popularity as a way of excluding oxygen from food and extending shelf lives.¹⁶

An ideal colorimetric sensor for real-time food spoilage would sense gaseous BAs at low concentrations and rapidly undergo a distinct color change at a reasonable reaction rate. Further, this sensor must have the ability to selectively detect the primary amines as well as the source of the degradation, oxygen gas. This ideal sensor would be composed of cheap, low-toxicity materials derived from edible sources and possess a minimal environmental footprint. We aimed to design a sensor satisfying as many of these criteria as possible.

Genipin, (Scheme 1, bottom) a naturally occurring, colorless iridoid, has found numerous applications in diverse fields, most commonly used as a non-toxic cross-linking reagent for biomaterials.^{17,18} As an edible fruit extract, genipin also has relatively low toxicity; a recent study shows that neither genipin nor the resultant gardenia blue dye exhibit genotoxicity.¹⁹ This transformation from a colorless iridoid to dye can be attributed to the fact that genipin reacts with amines through a double condensation reaction forming an oxidatively unstable

dihydropyridine intermediate (Scheme 1, bottom). In the presence of oxygen, this intermediate further undergoes oxidative oligomerization/polymerization resulting in a brilliant blue dye.²⁰ However, to the best of our knowledge genipin has yet to find applications as a sensor, despite this unique reactivity positioning it as an ideal candidate for a colorimetric sensor for food degradation.

We sought to study the ability of genipin to react with BAs and confirm the selectivity towards a broad range of amines. Herein, we describe the use of genipin as the first dual colorimetric sensor for both O₂ and BAs in solution and provide a proof of concept in an immobilized form.

Experimental Section

Materials. All chemical reagents or solvents were purchased from Fisher Scientific or Millipore Sigma and used as received unless otherwise indicated. Genipin was kindly donated by Inkbox Ink and purity verified independently by ¹H NMR analysis. Food-grade sodium alginate was purchased from BYOB Cocktail Emporium in Toronto and used without purification. NMR spectra were obtained at 25 °C on either a Bruker 700 MHz Spectrometer, Bruker DRX 600 MHz Spectrometer, Bruker ARX 400 MHz Spectrometer or, Bruker ARX 300 MHz Spectrometer. Chemical shifts are reported relative to SiMe₄ and referenced to the residual solvent signal (¹H, ¹³C{¹H}). NMR spectra were analyzed using either TopSpin 4.0.1 or MestReNova 6.0.2-5475 software. Chemical shifts are reported in ppm and coupling constants as scalar values in Hz. The conventional abbreviations were used as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet), br (broad). Absorption measurements were recorded with a Cary 5000 UV-vis-NIR Spectrophotometer from Agilent Technologies. Recordings were obtained at 25 °C and taken with the instrument operating in dual beam mode and referenced to methanol. All absorption experiments were conducted in quartz cuvettes (1cm x 1cm) equipped with a Teflon seal.

UV-visible absorbance detection of biogenic amines in solution 22.6 mg (0.10 mmol) of genipin was dissolved in 45.0 mL methanol in a single-neck round-bottom flask by stirring for five minutes with a magnetic stirrer. For 1:1 solutions of genipin:BA, 0.10 mmol of BA were added to a separate pre-weighed vial and rinsed into the genipin solution with a further 5.0 mL methanol to prepare 50.0 mL of solution of concentrations 2.0 x 10⁻³ M in genipin and BA. For solutions lower in concentration of BA, stock solutions 0.020 M in BA were prepared in methanol. Appropriate volumes of additional methanol were added as needed to dilute to desired concentrations, with total volumes made up to 50.0 mL. Round-bottomed flasks containing solutions were left open to allow exposure to oxygen and stirred for 48-72 hours. Measurement of UV-visible absorbances were carried out by decanting solutions into a graduated cylinder at time of measurement, making up the total volume to 50.0 mL with methanol to compensate for evaporation, decanting back into the round-bottom flask and stirring for 5 minutes to achieve homogeneity. Samples were then transferred into a quartz cuvette for analysis. Single-neck (1" diameter) round-bottom flasks were chosen as reaction vessels after verifying that rates of

evaporation of stirred methanol at room temperature ensured that solution volumes never fell below 40 mL during the course of the reaction.

Preparation of genipin-embedded calcium alginate gel beads. 1, 10, 50, or 100 mg of genipin was dissolved in 10.0 mL of distilled water in a 100 mL beaker. In the case of the addition of 100 mg of genipin, heating to 80 °C was required for full dissolution. 200 mg of sodium alginate powder was added with vigorous stirring to form the sodium alginate gel. Using a variation of the simple dripping (extrusion) technique described by Bhandari et. al.,²¹ a 1 mL disposable syringe without a needle was employed to uptake 1.00 mL of sodium alginate gel. Due to dripping of gel from the outside of the syringe, the first 1-2 drops were dispensed back into the gel mixture. The remainder was dispensed dropwise from a height of 10 cm above the surface of 25.0 mL of a 1.0 %w/v CaCl_2 solution contained in a 125 mL Erlenmeyer flask. 12-15 beads were dispensed in this manner until the syringe contained >0.1 mL of gel, at which point the syringe was re-filled to avoid size decrease of gel beads due to air bubbles inside the syringe, and lack of material. After 100-110 beads had been dispensed in this manner, using 75-80% of the sodium alginate gel mass, the remaining CaCl_2 solution was decanted from the beads and the beads were washed with 10 mL of an aqueous solution of 0.1 M $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ pH 4 buffer. Beads were then dispensed onto a watch glass, separated using tweezers and permitted to stand 1 hour to allow excess water to run onto the glass. The beads were then removed from the watch glass and stored in a sealed vial in a refrigerator at 4 °C.

General Procedure for reaction of genipin immobilized in calcium alginate gel beads with primary amine vapors. 1-10 genipin-embedded calcium alginate gel beads were placed in a 20 mL vial using a clean pair of tweezers. 0.10 mmol of primary amine was added to a 1-dram vial (or Eppendorf tube) which was placed, uncapped, inside the 4-dram vial containing the beads, and the larger vial was sealed with a cap and either left at room temperature or placed in a refrigerator at 4 °C and monitored visually for up to 48 hours days.

In the case where ten gel beads were exposed to 0.10 mmol amine, after 24 hours at room temperature the gel beads remained colourless, while after 48 hours all ten beads had turned blue. In the case where only one bead was used instead of ten beads, the single bead turned deep blue within 24 hours.

To achieve dissolution of gel beads to obtain UV-vis spectra after specified reaction times of 6-48 hours, 10 dyed genipin-containing gel beads were added to 3.5 mL of a 55 mM solution of sodium citrate to allow dissolution of gel beads by cation exchange mediated by the citrate cation chelator.²² Although some of the blue dye became insoluble particulate in this aqueous solution, precluding qualitative UV-vis absorption measurements, enough remained in solution to qualitatively observe the blue dye by UV-vis spectroscopy.

Reaction of genipin immobilized in calcium alginate gel beads with raw chicken. 10 genipin-embedded calcium alginate gel beads produced no more than seven days previously were placed in each of two 20 mL vials using a clean pair of tweezers. A piece of chicken breast

purchased within the previous 24 hours from a commercial grocery store was cut into slices of mass 5-6 grams and one slice was placed in each vial using a separate, clean pair of tweezers, such that the gel beads were separated from the surface of the raw chicken. The vials were sealed with a plastic screw cap. One was left at room temperature, while one was refrigerated at 4 °C. Both samples were monitored visually and photographed at hours 1, 2, 3 and 6.

Results and Discussion

To confirm the selectivity of genipin for primary amines, the reactions with a series of amines in methanol solutions were monitored over periods of 48-72 hours at room temperature. These reactions were performed at concentrations of 2.0×10^{-3} M for both genipin and amine, in open vessels to permit diffusion of O_2 into the system to promote blue dye formation. Benzylamine, *N*-methylbenzylamine, and *N,N*-dimethylbenzylamine were initially tested to represent a series of simple, analogous primary, secondary and tertiary amines, and reactions were monitored by UV-vis absorbance spectroscopy. Consistent with reported literature, formation of blue dye was only observed in the reaction of genipin with the primary benzylamine, with strong visible absorbance exhibited between 550-650 nm (Figure S4). Though solutions of tertiary and secondary amines, *N,N*-dimethylbenzylamine and *N*-methylbenzylamine, did exhibit a colour change to reddish golden, they did not form the blue dye even after extended reaction times. Monitoring the reactions of *N,N*-dimethylbenzylamine and *N*-methylbenzylamine, by 1H NMR spectroscopy with genipin at greater concentrations does indicate a reaction between genipin and amine but it produced an intractable mixture of products. These studies unequivocally show the selectivity of genipin to react with primary amines to form a blue product.

Reactions with biogenic amines putrescine, cadaverine, tyramine and histamine also exhibited formation of deep blue dye (Figure 1). In these cases, an incubation period of 12-24 hours was required before strong absorbances with maxima at 590-610 nm were observed. The incubation time is attributable to the time required for growth of conjugated oligomers absorbing in the red region. Although reactions were monitored for up to 120 hours, in all cases, λ_{max} and molar attenuation coefficient values demonstrated only minimal change after 48 hours (e.g. Figure S5). Interestingly, molar attenuation coefficients varied widely between reactions of genipin with different BA, ranging from 1.2×10^2 Lmol $^{-1}$ cm $^{-1}$ in putrescine to 4.4×10^3 Lmol $^{-1}$ cm $^{-1}$ in tyramine. It is not obvious from differences in molecular structure of the amines why significant variance is observed in molar attenuation coefficients of the solutions of dyes.

Figure 1 - Stacked UV-vis Spectra for the reaction of genipin with BAs. Dilution factors - 1,4-diaminobutane (no dilution); 1,5-diaminopentane (5x dilution); Tyramine (10x dilution); Histamine (20x dilution).

Unsurprisingly, when reactions were carried out at increased concentrations of genipin and amine, the blue dye formed much more rapidly, for example within 4 hours at concentrations of 0.20 M (Figure S10)).

From these solution studies, the limit of visual detection of amines with a 0.50 M solution of genipin in 50:50 methanol:water was found to vary depending on BA used. Putrescine and tyramine, the tested amines whose dyes exhibited the lowest and highest molar attenuation coefficients, respectively, were examined. Solutions of genipin in methanol exhibit a dull golden tinge, while presence of the blue dyes were confirmed visually by a change in colour to dull green, the result of the presence of both blue dye and the golden hue from remaining unreacted genipin. Tyramine could be visually detected at concentrations of 6.9 ppm after 24 hours (Figure S27), while the detection limit for putrescine was nearly an order of magnitude higher at 44 ppm after 24 hours. This trend correlates with the measured trend in molar attenuation coefficient measured for the dyes incorporating putrescine or tyramine units (converted from molarity).

Enticingly, the reaction of putrescine with genipin in the absence of oxygen produced a different response (Figure 2). Using degassed water and an atmosphere of nitrogen, the solutions produced a golden red coloration with no indication of the blue response as indicated by the λ_{max} at ~600 nm. Upon exposure to air after 48 hours, the solutions rapidly turned blue. To explore the sensitivity of the genipin and amine intermediate to oxygen gas, we found even 0.5% (5000 ppm) of oxygen gas (converted from moles of H_2O_2 , see SI) added under an

atmosphere of N_2 were enough to trigger the oxidative oligomerization reaction turning the sample blue over a period of 24 hours. This sensitivity is below the oxygen threshold established in modified atmospheric packaging ($\sim 2\%$) and in the desired range of sensitivity ($0.5\text{-}2\%$ O_2 level).¹⁶

Figure 2 - UV-vis absorbance spectra of 2.0 mM solutions of genipin in methanol in reaction with 2.0mM of 1,4-diaminobutane in the presence (blue) and absence (red) of O_2 after 48 hours.

To demonstrate the applicability of our sensing platform for food spoilage detection, an immobilization method for genipin was developed using a calcium alginate gel matrix. Alginate gels are common edible food thickening agents derived from alginic acid sourced from seaweed or brown algae.²² Calcium alginate gel beads are easily produced from sodium alginate and permit penetration of small molecules through their pores.²¹ Genipin was embedded in these beads through modifying a literature procedure for the impregnation of calcium alginate gel beads with enzymes.²¹ After extensive screening, it was found that a concentration of 5 mg/mL of genipin in water, approximately equivalent to the room-temperature solubility of genipin in water, resulted in the most responsive beads. As a proof of concept, we selected the most volatile biogenic amine, putrescine, to test the sensing capabilities of the beads. Immobilized genipin-containing beads were exposed to the gaseous BA by adding 0.1 mmol of putrescine to an open 1-dram vial and sealing the 1-dram vial inside a 4-dram vial containing 10 genipin-infused beads. The beads responded as expected and a blue colour developed upon exposure to BA vapors within 24 hours (Figure 3). To mimic conditions for food storage, these experiments were also undertaken at 4 °C, and encouragingly the beads did not exhibit any color change, even after 48 hours (Figure S28). Gel beads could subsequently be dissolved in an aqueous citric acid solution for UV-vis absorbance analysis (Figure 3), although undissolved particulate matter interfered with quantitative measurements.

In analyzing the absorbance of solutions obtained from dissolving gel beads exposed for 48 hours to vials containing varying amounts of putrescine (Figure S29), it is evident that increasing

amounts of amine result in increased intensity of the blue dye. The vapor pressure of linear putrescine has been reported as 259.3 Pa at 300.7K.²³ Using the reported vapor pressure at 300.7 K as an approximation for the vapor pressure at room temperature, it can be calculated from the ideal gas law that for the 18 mL closed volume of the 4-dram vial, evaporation of <0.2 mg of putrescine is required to reach equilibrium vapor pressure. Upon dissolution after reaction, solutions of gel beads exposed to 0.3 mg, 1.2 mg and 12 mg of putrescine in open one-dram vials demonstrated significantly increased absorbance as the amount of amine was increased (Figure S29). Thus, it can be hypothesized that the intensity of the absorbance is more correctly correlated to the absolute amount of amine available for reaction, and the identity of that amine, than the vapor pressure.

Figure 3: Genipin-impregnated beads after 24 hours exposure to putrescine vapours at room temperature

Having established the sensor's response to gaseous amines, we sought to test them against real food samples. Samples of chicken breast (5-6 grams each) were freshly prepared and stored in sealed vials with the genipin-impregnated beads. With the beads in close proximity to the meat, the sensors behaved as expected and turned blue upon exposure. Samples were stored both at room temperature and in a refrigerator at 4 °C to mimic typical storage conditions. To quantify the color difference between the beads, ΔE^* values were determined following commission internationale de l'éclairage (CIE) guidelines (Figure 4). A ΔE^* value >5 is considered to be distinguishable by the naked eye.¹² This approach has previously been used to determine

the effectiveness of small molecule amine sensors.¹² The sensors were monitored over the course of 6 hours with images taken at time points of 1, 2, 3 and 6 hours (Table S1). ΔE^* values of 38 and 0 were obtained after 6 hours for the samples at room temperature and at 4 °C, respectively. These values correspond to a significant blue coloration of the beads at room temperature versus no visible change in the refrigerator (Figure 4). The rate of colour appearance in these indicator beads is consistent with the USDA health advisory on raw poultry, which warns against leaving raw poultry at room temperature for longer than two hours.²⁴

Figure 4: Change in color of Genipin Impregnated Beads in the presence of chicken, room temperature (blue) and 4 °C (orange).

Conclusion

In summary, this proof-of-principal study indicates that genipin can act as a highly selective dual sensor for both biogenic amines and O_2 . This uniquely positions genipin as an ideal colorimetric sensor for food spoilage as it can detect the root cause of this process through off-gases. Moreover, by immobilizing the sensor in an edible calcium alginate matrix, biogenic amine vapors were detected at very low concentrations. This was extended to monitoring chicken spoilage by the naked eye, indicating its potential in food spoilage detection.

Acknowledgement

The authors gratefully acknowledge financial support from NSERC of Canada, the Canadian Foundation for Innovation and Inkbox Ink. CBC is grateful for the award of a Canada Research Chair. The authors thank Professor Thomas Baumgartner for the use of the UV-vis spectrophotometer and valuable feedback.

References

1. Santos MHS. Biogenic amines: their importance in foods. *Int J Food Microbiol.* 29:213–31 (1996);
2. Önal A. A review: Current analytical methods for the determination of biogenic amines in foods. *Food Chem.* 103:1475–86 (2007);

3. Naila A, Flint S, Fletcher G, Bremer P, Meerdink G. Control of Biogenic Amines in Food-Existing and Emerging Approaches. *J Food Sci.* 75:R139–50 (2010);
4. Liu SF, Petty AR, Sazama GT, Swager TM. Single-Walled Carbon Nanotube/Metalloporphyrin Composites for the Chemiresistive Detection of Amines and Meat Spoilage. *Angew. Chem. Int Ed.* 54:6554–7 (2015);
5. Aschemann-Witzel J. Waste not, want not, emit less: Reducing food waste in the supply chain and at home can help to reduce carbon emissions. Vol. 352, *Science.* 352:408–9 (2016);
6. Sotzing GA, Phend JN, Grubbs RH, Lewis NS. Highly Sensitive Detection and Discrimination of Biogenic Amines Utilizing Arrays of Polyaniline/Carbon Black Composite Vapor Detectors. *Chem Mater.* 12:593–5 (2000);
7. Yousefi H, Ali MM, Su H-M, Filipe CDM, Didar TF. Sentinel Wraps: Real-Time Monitoring of Food Contamination by Printing DNAzyme Probes on Food Packaging. *ACS Nano.* 12:3287–94 (2018);
8. Barandun G, Soprani M, Naficy S, Grell M, Kasimatis M, Chiu KL, Ponzoni A, Güder F. Cellulose Fibers Enable Near-Zero-Cost Electrical Sensing of Water-Soluble Gases. *ACS Sensors.* 4:1662–9 (2019);
9. Chopra S, Singh A, Venugopalan P, Singh N, Kaur N. Organic nanoparticles for visual detection of spermidine and spermine in vapors and aqueous phase. *ACS Sustain Chem Eng.* 5:1287–96 (2017)
10. Valderrey V, Bonasera A, Fredrich S, Hecht S. Light-Activated Sensitive Probes for Amine Detection. *Angew. Chem. Int Ed.* 56:1914–8 (2017);
11. Li L, Li W, Ran X, Wang L, Tang H, Cao D. A highly efficient, colorimetric and fluorescent probe for recognition of aliphatic primary amines based on a unique cascade chromophore reaction. *Chem Commun.* 55:9789–92 (2019);
12. Diaz YJ, Page ZA, Knight AS, Treat NJ, Hemmer JR, Hawker CJ, Read De Alaniz J. A Versatile and Highly Selective Colorimetric Sensor for the Detection of Amines. *Chem. Eur J.* 23:3562–6 (2017);
13. Chen Q, Diaz YJ, Hawker MC, Martinez MR, Page ZA, Xiao-An Zhang S, Hawker CJ, Read de Alaniz J. Stable Activated Furan and Donor-Acceptor Stenhouse Adduct Polymer Conjugates as Chemical and Thermal Sensors. *Macromolecules.* 52:4370–5 (2019);
14. Dudnyk I, Janeček E-R, Vaucher-Joset J, Stellacci F. Edible sensors for meat and seafood freshness. *Sensors Actuators B Chem.* 259:1108–12 (2018);
15. Huang X, Zou X, Shi J, Guo Y, Zhao J, Zhang J, Hao L. Determination of pork spoilage by colorimetric gas sensor array based on natural pigments. *Food Chem.* 145:549–54 (2014);
16. Mills A. Oxygen indicators and intelligent inks for packaging food. *Chem. Soc. Rev.* 34:1003–11 (2005).

17. Yan LP, Wang YJ, Ren L, Wu G, Caridade SG, Fan JB, Wang LY, Ji PH, Oliveira JM, Oliveira JT, Mano JF, Reis RL. Genipin-cross-linked collagen/chitosan biomimetic scaffolds for articular cartilage tissue engineering applications. *J Biomed Mater Res Part A*. 95A:465–75 (2010);
18. Butler MF, Ng Y-F, Pudney PDA. Mechanism and kinetics of the crosslinking reaction between biopolymers containing primary amine groups and genipin. *J Polym Sci Part A Polym Chem*. 41:3941–53 (2003);
19. Tsai C-C, Huang R-N, Sung H-W, Liang HC. In vitro evaluation of the genotoxicity of a naturally occurring crosslinking agent (genipin) for biologic tissue fixation. *J Biomed Mater Res*. 52:58–65 (2000);
20. Touyama R, Takeda Y, Inoue K, Kawamura Ik, Yatsuzuka M, Ikumoto T, Shingu T, Yokoi T, Inouye H. Studies on the Blue Pigments Produced from Genipin and Methylamine. I. Structures of the Brownish-Red Pigments, Intermediates Leading to the Blue Pigments. *Chem Pharm Bull*. 42:668–73 (1994);
21. Ching SH, Bansal N, Bhandari B. Alginate gel particles—A review of production techniques and physical properties. *Crit Rev Food Sci Nutr*. 57:1133–52 (2017);
22. Goh CH, Heng PWS, Chan LW. Alginates as a useful natural polymer for microencapsulation and therapeutic applications. Vol. 88, Carbohydrate Polymers. p. 1–12 2012.
23. Pozdeev VA, Verevkin SP. Vapor pressure and enthalpy of vaporization of linear aliphatic alkanediamines. *J Chem Thermodyn*. 43:1791–9 (2011);
24. https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/keep-food-safe-food-safety-basics/ct_index

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