engineering proceedings

MDPI

Proceedings

Inclusion of Natural Anthocyanins as Food Spoilage Sensors †

M. Carpena [1], Aurora Silva[1,2] , P. Barciela [1], A. Perez-Vazquez [1], F. Chamorro [1], L. Cassani [1], M. Fátima Barroso[2,*], Jianbo Xiao [1], M.A. Prieto [1,*] and J. Simal-Gandara [1]

1. Universidade de Vigo, Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Faculty of Science, E32004 Ourense, Spain. mcarpena@uvigo.es (M.C.); pau_barci@hotmail.es (P.B.); anaperezvazquez00@gmail.com (A.P.-V.); franklin.noel.chamorro@uvigo.es (F.C.); luciavictoria.cassani@uvigo.es (L.C.); mprieto@uvigo.es (M.A.P.); jianbo.xiao@uvigo.es (J.X.); jsimal@uvigo.es (J.S.-G.)
2. REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr António Bernardino de Almeida 431, 4200-072 Porto, Portugal. mass@isep.ipp.pt (A.S.); mfb@isep.ipp.pt (M.F.-B.).

* Correspondence: mprieto@uvigo.es (M.A.P.); mfb@isep.ipp.pt (M.F.-B.).

† Presented at the title, place, and date.

Abstract: Food safety is one of the most up-to-date subjects under the scope of the scientific community since it is a fundamental issue for the general population. The desire to use a simple, inexpensive, easy-to-read package freshness indicator led to a multitude of proposals for package real-time sensors for food freshness indicators. The sensors' design strategy is to target a physical or chemical modification that occurs by the spoiling process, such as changes in temperature, moisture or the detection of foodborne pathogens. One of the most common approaches is evaluating changes in pH since a significant amount of food spoilage occurs with significative alteration (e.g., acidity increases on dairy products). However, some safety concerns emerge from the use of complex artificial chemical molecules as pH indicators in active labels/packages for food. Natural occurring anthocyanins are a safe alternative to classic pH indicators and have been applied as sensitive molecules for pH changes aimed at the development of active labels and active packing for food. This proposal briefly reviews the latest scientific contributions on the application of anthocyanins in food spoilage sensors.

Keywords: anthocyanins; food spoilage; pH indicators; sensors

Citation: M. Carpena; Silva, A.; P. Barciela; A. Perez-Vazquez; F. Chamorro; L. Cassani; Barroso, F.; Xiao, J.; M.A. Prieto; J. Simal-Gandara Inclusion of Natural Anthocyanins as Food Spoilage Sensors. **2023**, *4*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Received: date
Accepted: date
Published: date

1. Introduction

The concern over the safety, quality, and shelf life of food goods is on the rise, leading to an important research line on biodegradable packaging or sensors offering shelf-life information. Intelligent packaging is defined as packaging that contains something (molecule, device, sensor, etc.) to monitor the condition of packaged food or the environment surrounding the food [1] and is activated by external stimuli (pH, water, or O_2) and can monitor and alert consumers on the state of the food in real-time.

Among the target parameters that can be used as an indicator of food freshness, pH stands out because it is affected by various types of food spoilage, such as excessive ripening of fruits and vegetables, decomposition of meat or fish due to decay of molecules such as lipids and proteins, causing variations in chemicals such as trimethylamine , dimethylamine, ammonia, hydrogen sulfide, carbon dioxide, [2] that lead to pH changes.[3]. Bacterial growth is also an important vector of spoilage [4] and can also be monitored by pH changes, for instance due to the dissoluble CO_2 [5] or to the increase of the lactic or acetic acids concentration. Furthermore, targeting pH as freshness indicator allows the creation of colorimetric sensors that could be included in the packaging and that provides real-time information in an easy way to consumers understand way, on food quality and safety [6].

Although the classical molecules like methyl red and Nile red [7], brilliant blue ([8], or meta-cresol purple sodium salt [9] are important in the development of pH-dependent color sensors, the appetence for natural and safer products directed the scientific community to turn to the polyphenols group, with especial focus in anthocyanins, plant-derived pigments with potential health benefits [10]. The ability of anthocyanin to change color in response to changes in pH is consequently applied to systems and sensors to monitor food quality, estimate food shelf life, and eventually employ color indicators in food packaging [11]. In this work, the use of anthocyanins-based sensors is briefly reviewed.

2. Discussion

The ability of anthocyanins to change color in a pH-dependent process has been exploited to fabricate sensors that can measure the pH variation of packaged foods serving as freshness indicators. **Table 1** presents a selection of the latest publications concerning smart packaging using this type of sensors.

Table 1. Selected studies of color-based sensors for food packaging applications using anthocyanins as sensitive molecule.

Anthocyanins Source	Base material /Technique	PH /NH_3	Color Change	Application	Ref.
Black carrot	Cellulose acetate	5.9-7.1	Pink to purple	Meat	[12]
	Cellulose nanofibers	6.36-7.22	Deep carmine to khaki	Fish	[13]
Clitoria ternatea	Polymeric film: Sago (*Metroxylon sagu*)	6.50-10.30	Blue to green	Chicken	[14]
	Bilayer film polycaprolactone nanofibers	7.10-7.35	Pale blue to yellow-green	Shrimp	[15]
Curcumin	Low density polyethylene film	5.95 -29.31 mg TVB-A/100 g	Yellow to brown	Meat/fish	[16]
	Matrix: corn starch, and polyvinyl alcohol	8. 06-8.41-8.8	Yellow to red	Fish	[17]
	Polylactic acid and polypropylene carbonate film	-	Yellow to light orange	Shrimps	[18]
	Nanofiber: chitosan and polyethylene oxide	6.20-6.75	Yellow to orange	Chicken	[19]
Hibiscus rosa sinensis	Fiber mats: polycaprolactone and polyethylene oxide +silver nanoparticles	6.40-8.06 (at 4ºC)	Beije to olive green	Shrimp	[20]
Grape skin	Gelatin-based films and mats	13.32–27.81 mg TVB-N	-	Meat	[21]
Malva silvestris	Matrix: polylactic acid / polyethylene glycol /calcium bentonite	6.5-8.0	Greenish yellow to pink	Minced meat/ chicken/ shrimp/ fish	[22]
Melastoma Malabathricum	Cellulose nanofibers and cellulose acetate	0.1-25 % NH_3	Pink to intense red to light pink to brown to dark green	Fish/meat	[23]
Mulberry	Bilayer films: gelatin, ZnO nanoparticles and gellant gum	4.7-20-7 mg /100g TVB-N	Pink to yellow	Fish	[24]
Purple Cabbage	Natural printed paper: hydroxyethyl cellulose, glycerol.	6.0-7.3	Purplish red to purple to bluish violet	Fish	[25]
Purple sweet potato	Double layer: internal hydrogel water, agar anthocyanins,	0.45 -35.92 mg /100g TVB-N	Dark pink to blue	Meat	[26]

	external sunflower oil, beeswax, and glyceride monooleate				
Red cabbage	Biocomposite membrane: chitosan and starch in acetic acid	2.5-5.6	Yellow to reddish-brown	Milk	[27]
	Nanocomposite films: polyvinyl alcohol and nanoclays	~3-~6-~8	Red to light pink to olive green	Shrimp	[28]
	Pectin-based film	50-300 TVB-N ppm	Purple pink to light pink to green-blue	Fish/meat	[29]
	Potato dextrose agar and starch	-	Brownish pink to yellowish green	Fish	[30]
	Modified cassava starches	2-12	Pink to purple to blue to green to yellow	-	[31]
Rosehip	Hydrogel: dimethylacrylamide, gelatin, citric acid	2-12	Red to purple	Milk/cheese	[32]
Roselle	Film: Polyvinylidene Fluoride, anthocyanins Cinnamon essential oil	5.6-7.4	Pink to blue	Pork meat	[33]
	Bilayer: Polyvinylidene Fluoride +polyvinyl alcohol/Sodium alginate	8.72-18.02 mg/100g TVB-N	Pink to bluish, then green to yellow	Pork meat	[34]

TVB-N -total volatile basic nitrogen; TVB-A Total volatile basic amines.

The inclusion of vegetal pigments as pH indicators such as anthocyanins compounds have several advantages since these molecules are safe, biocompatible and present a series of very important biological and functional properties, including antioxidant activity, free radical scavenging activity, antimicrobial activity, among others [35]. Additionally, anthocyanins are known to provide benefits for the immune system, cardiovascular health, and prevent obesity by suppressing digestive enzymes [36–38]**.** Furthermore, anthocyanins have also been used in clinical tests, showing ability to reduce the in oxidative stress and the inflammatory indicators, as well as presented a positive impact on vascular function and hyperlipidemia. Moreover, these molecules might have an impact on glucose homeostasis and cognitive impairment [36].

However, an important drawback of anthocyanins is their lack of stability. Thus, a variety of elements, including pH, light, temperature, oxygen, and enzymes, have an impact on their stability[39]. Furthermore, the temperature, a crucial parameter in food processing, affects particularly heat-sensitive molecules such as anthocyanins [40]. So, it is important to highlight research works where the anthocyanins stability is a special focus. In a recent study, ZhikunYang *et al.*, developed a by-layer sensor by including ZnO nanoparticles in a gelatin-based layer. They achieved an increase in the color stability of the mulberry fruit anthocyanins with a limit of detection of 0.01 mM of NH_3 [24]. In another work, the use of polycaprolactone nanofibers also led to color stability and the color variation was reversible, which open the possibility of reuse the material [15].

Likewise, several reports showed strategies to improve the anthocyanins- based sensors physical properties by improving the thermal stability [26], water resistance and vapor transmission [27] water resistance and tensile strength [31]. Additionally, strategies such as inclusion of cellulose nanofiber enhance the biodegradability of active packaging materials [23].Up- to- date, research items showed that this area of study is of crucial importance, pursuing safer, intuitive, and biocompatible ways of informing consumers of the freshness state of perishable products foods.

Author Contributions: Conceptualization, A.S. and M.C.; methodology P.B. and A.P.-V., investigation F.C. and L.C.; resources M.F.B., J.X., J.S.-G. and M.A.P.; writing—original draft preparation A.S. and M.C.; writing—review and editing A.S., M.C. and L.C.; visualization, J.S.-G., M.F.B.;

supervision J.S.-G., and M.A.P., project administration M.A.P. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: The research leading to these results was supported by MICINN supporting the Ramón y Cajal grant for Jianbo Xiao (RYC-2020-030365-I) and M.A. Prieto (RYC-2017-22891); by Xunta de Galicia for supporting the program EXCELENCIA-ED431F 2020/12 that supports the work of F. Chamorro, the post-doctoral grant of L. Cassani (ED481B-2021/152), and the pre-doctoral grant of M. Carpena (ED481A 2021/313). The JU receives support from the European Union's Horizon 2020 research and innovation program and the Bio Based Industries Consortium. The project SYSTEMIC Knowledge hub on Nutrition and Food Security, has received funding from national research funding parties in Belgium (FWO), France (INRA), Germany (BLE), Italy (MIPAAF), Latvia (IZM), Norway (RCN), Portugal (FCT), and Spain (AEI) in a joint action of JPI HDHL, JPI-OCEANS and FACCE-JPI launched in 2019 under the ERA-NET ERA-HDHL (n° 696295). The authors would like to thank the EU and FCT for funding through the programs UIDB/50006/2020; UIDP/50006/2020; LA/P/0008/2020 and to Ibero-American Program on Science and Technology (CYTED— GENOPSYSEN, P222RT0117). Fatima Barroso (2020.03107.CEECIND) thanks FCT for the FCT Investigator grant.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Müller, P.; Schmid, M. Intelligent Packaging in the Food Sector: A Brief Overview. *Foods* **2019**, *8*, 16, doi:10.3390/foods8010016.
2. Liu, D.; Zhang, C.; Pu, Y.; Chen, S.; Liu, L.; Cui, Z.; Zhong, Y. Recent Advances in pH-Responsive Freshness Indicators Using Natural Food Colorants to Monitor Food Freshness. *Foods* **2022**, *11*, 1884, doi:10.3390/foods11131884.
3. Cheng, H.; Xu, H.; Julian McClements, D.; Chen, L.; Jiao, A.; Tian, Y.; Miao, M.; Jin, Z. Recent advances in intelligent food packaging materials: Principles, preparation and applications. *Food Chem.* **2022**, *375*, 131738, doi:10.1016/j.foodchem.2021.131738.
4. Betts, G.D.; Linton, P.; Betteridge, R.J. Food spoilage yeasts: effects of pH, NaCl and temperature on growth. *Food Control* **1999**, *10*, 27–33, doi:10.1016/S0956-7135(98)00151-0.
5. Puligundla, P.; Jung, J.; Ko, S. Carbon dioxide sensors for intelligent food packaging applications. *Food Control* **2012**, *25*, 328–333, doi:10.1016/j.foodcont.2011.10.043.
6. Alizadeh-Sani, M.; Mohammadian, E.; Rhim, J.-W.; Jafari, S.M. pH-sensitive (halochromic) smart packaging films based on natural food colorants for the monitoring of food quality and safety. *Trends Food Sci. Technol.* **2020**, *105*, 93–144, doi:10.1016/j.tifs.2020.08.014.
7. Chen, Y.; Fu, G.; Zilberman, Y.; Ruan, W.; Ameri, S.K.; Zhang, Y.S.; Miller, E.; Sonkusale, S.R. Low cost smart phone diagnostics for food using paper-based colorimetric sensor arrays. *Food Control* **2017**, *82*, 227–232, doi:10.1016/j.foodcont.2017.07.003.
8. Lee, K.; Meng, X.; Kang, T.-Y.; Ko, S. A dye-incorporated chitosan-based CO2 indicator for monitoring of food quality focusing on makgeolli quality during storage. *Food Sci. Biotechnol.* **2015**, *24*, 905–912, doi:10.1007/s10068-015-0117-9.
9. Perez de Vargas-Sansalvador, I.M.; Erenas, M.M.; Diamond, D.; Quilty, B.; Capitan-Vallvey, L.F. Water based-ionic liquid carbon dioxide sensor for applications in the food industry. *Sensors Actuators B Chem.* **2017**, *253*, 302–309, doi:10.1016/j.snb.2017.06.047.
10. Khoo, H.E.; Azlan, A.; Tang, S.T.; Lim, S.M. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr. Res.* **2017**, *61*, 1361779, doi:10.1080/16546628.2017.1361779.
11. Oladzadabbasabadi, N.; Mohammadi Nafchi, A.; Ghasemlou, M.; Ariffin, F.; Singh, Z.; Al-Hassan, A.A. Natural anthocyanins: Sources, extraction, characterization, and suitability for smart packaging. *Food Packag. Shelf Life* **2022**, *33*, 100872, doi:10.1016/J.FPSL.2022.100872.
12. Franco, M.R.; da Cunha, L.R.; Bianchi, R.F. Janus principle applied to food safety: An active two-faced indicator label for tracking meat freshness. *Sensors Actuators B Chem.* **2021**, *333*, 129466, doi:10.1016/j.snb.2021.129466.
13. Moradi, M.; Tajik, H.; Almasi, H.; Forough, M.; Ezati, P. A novel pH-sensing indicator based on bacterial cellulose nanofibers and black carrot anthocyanins for monitoring fish freshness. *Carbohydr. Polym.* **2019**, *222*, 115030, doi:10.1016/j.carbpol.2019.115030.
14. Ahmad, A.N.; Abdullah Lim, S.; Navaranjan, N. Development of sago (Metroxylon sagu)-based colorimetric indicator incorporated with butterfly pea (Clitoria ternatea) anthocyanin for intelligent food packaging. *J. Food Saf.* **2020**, *40*, doi:10.1111/jfs.12807.
15. Liu, L.; Zhang, J.; Zou, X.; Arslan, M.; Shi, J.; Zhai, X.; Xiao, J.; Wang, X.; Huang, X.; Li, Z.; et al. A high-stable and sensitive colorimetric nanofiber sensor based on PCL incorporating anthocyanins for shrimp freshness. *Food Chem.* **2022**, *377*, 131909, doi:10.1016/j.foodchem.2021.131909.
16. Zhai, X.; Wang, X.; Zhang, J.; Yang, Z.; Sun, Y.; Li, Z.; Huang, X.; Holmes, M.; Gong, Y.; Povey, M.; et al. Extruded low density polyethylene-curcumin film: A hydrophobic ammonia sensor for intelligent food packaging. *Food Packag. Shelf Life* **2020**, *26*, 100595, doi:10.1016/j.fpsl.2020.100595.

17. Liu, D.; Dang, S.; Zhang, L.; Munsop, K.; Li, X. Corn starch/polyvinyl alcohol based films incorporated with curcumin-loaded Pickering emulsion for application in intelligent packaging. *Int. J. Biol. Macromol.* **2021**, *188*, 974–982, doi:10.1016/j.ijbiomac.2021.08.080.
18. Cvek, M.; Paul, U.C.; Zia, J.; Mancini, G.; Sedlarik, V.; Athanassiou, A. Biodegradable Films of PLA/PPC and Curcumin as Packaging Materials and Smart Indicators of Food Spoilage. *ACS Appl. Mater. Interfaces* **2022**, *14*, 14654–14667, doi:10.1021/acsami.2c02181.
19. Yildiz, E.; Sumnu, G.; Kahyaoglu, L.N. Monitoring freshness of chicken breast by using natural halochromic curcumin loaded chitosan/PEO nanofibers as an intelligent package. *Int. J. Biol. Macromol.* **2021**, *170*, 437–446, doi:10.1016/j.ijbiomac.2020.12.160.
20. Jovanska, L.; Chiu, C.-H.; Yeh, Y.-C.; Chiang, W.-D.; Hsieh, C.-C.; Wang, R. Development of a PCL-PEO double network colorimetric pH sensor using electrospun fibers containing Hibiscus rosa sinensis extract and silver nanoparticles for food monitoring. *Food Chem.* **2022**, *368*, 130813, doi:10.1016/j.foodchem.2021.130813.
21. Pereira, P.F.M.; de Sousa Picciani, P.H.; Calado, V.; Tonon, R. V. Gelatin-based films and mats as electro-sensoactive layers for monitoring volatile compounds related to meat spoilage. *Food Packag. Shelf Life* **2023**, *36*, 101049, doi:10.1016/j.fpsl.2023.101049.
22. Ghorbani, M.; Divsalar, E.; Molaei, R.; Ezati, P.; Moradi, M.; Tajik, H.; Abbaszadeh, M. A halochromic indicator based on polylactic acid and anthocyanins for visual freshness monitoring of minced meat, chicken fillet, shrimp, and fish roe. *Innov. Food Sci. Emerg. Technol.* **2021**, *74*, 102864, doi:10.1016/j.ifset.2021.102864.
23. Hazarika, K.K.; Konwar, A.; Borah, A.; Saikia, A.; Barman, P.; Hazarika, S. Cellulose nanofiber mediated natural dye based biodegradable bag with freshness indicator for packaging of meat and fish. *Carbohydr. Polym.* **2023**, *300*, 120241, doi:10.1016/j.carbpol.2022.120241.
24. Yang, Z.; Zhai, X.; Zou, X.; Shi, J.; Huang, X.; Li, Z.; Gong, Y.; Holmes, M.; Povey, M.; Xiao, J. Bilayer pH-sensitive colorimetric films with light-blocking ability and electrochemical writing property: Application in monitoring crucian spoilage in smart packaging. *Food Chem.* **2021**, *336*, doi:10.1016/j.foodchem.2020.127634.
25. Sun, Y.; Wen, J.; Chen, Z.; Qiu, S.; Wang, Y.; Yin, E.; Li, H.; Liu, X. Non-destructive and Rapid Method for Monitoring Fish Freshness of Grass Carp Based on Printable Colorimetric Paper Sensor in Modified Atmosphere Packaging. *Food Anal. Methods* **2022**, *15*, 792–802, doi:10.1007/s12161-021-02158-2.
26. Guo, M.; Wang, H.; Wang, Q.; Chen, M.; Li, L.; Li, X.; Jiang, S. Intelligent double-layer fiber mats with high colorimetric response sensitivity for food freshness monitoring and preservation. *Food Hydrocoll.* **2020**, *101*, 105468, doi:10.1016/j.foodhyd.2019.105468.
27. Rai, P.; Verma, S.; Mehrotra, S.; Priya, S.; Sharma, S.K. Sensor-integrated biocomposite membrane for food quality assessment. *Food Chem.* **2023**, *401*, 134180, doi:10.1016/j.foodchem.2022.134180.
28. Abu-Thabit, N.; Hakeem, A.S.; Mezghani, K.; Ratemi, E.; Elzagheid, M.; Umar, Y.; Primartomo, A.; Al Batty, S.; Azad, A.K.; Al Anazi, S.; et al. Preparation of pH-Indicative and Flame-Retardant Nanocomposite Films for Smart Packaging Applications. *Sensors* **2020**, *20*, 5462, doi:10.3390/s20195462.
29. Dudnyk, I.; Janeček, E.-R.; Vaucher-Joset, J.; Stellacci, F. Edible sensors for meat and seafood freshness. *Sensors Actuators B Chem.* **2018**, *259*, 1108–1112, doi:10.1016/j.snb.2017.12.057.
30. Devarayan, K.; Motcham, V.V.; Kathavarayan, M.; Anjappan, H. Real-Time Detection of Packaged Seer Fish Spoilage Using Halochromic Optical Nose. *J. Aquat. Food Prod. Technol.* **2021**, *30*, 484–495, doi:10.1080/10498850.2021.1897049.
31. Cheng, M.; Cui, Y.; Yan, X.; Zhang, R.; Wang, J.; Wang, X. Effect of dual-modified cassava starches on intelligent packaging films containing red cabbage extracts. *Food Hydrocoll.* **2022**, *124*, 107225, doi:10.1016/j.foodhyd.2021.107225.
32. Ersen Dudu, T. Novel dimethylacrylamide/gelatin/rosehip based smart hydrogels as a color sensor in food packaging materials. *Asia-Pacific J. Chem. Eng.* **2023**, *18*, doi:10.1002/apj.2868.
33. Zhang, J.; Huang, X.; Zhang, J.; Liu, L.; Shi, J.; Muhammad, A.; Zhai, X.; Zou, X.; Xiao, J.; Li, Z.; et al. Development of nanofiber indicator with high sensitivity for pork preservation and freshness monitoring. *Food Chem.* **2022**, *381*, 132224, doi:10.1016/j.foodchem.2022.132224.
34. Zhang, J.; Huang, X.; Shi, J.; Liu, L.; Zhang, X.; Zou, X.; Xiao, J.; Zhai, X.; Zhang, D.; Li, Y.; et al. A visual bi-layer indicator based on roselle anthocyanins with high hydrophobic property for monitoring griskin freshness. *Food Chem.* **2021**, *355*, 129573, doi:10.1016/j.foodchem.2021.129573.
35. Cisowska, A.; Wojnicz, D.; Hendrich, A.B. Anthocyanins as Antimicrobial Agents of Natural Plant Origin. *Nat. Prod. Commun.* **2011**, *6*, doi:10.1177/1934578X1100600136.
36. Ockermann, P.; Headley, L.; Lizio, R.; Hansmann, J. A Review of the Properties of Anthocyanins and Their Influence on Factors Affecting Cardiometabolic and Cognitive Health. *Nutrients* **2021**, *13*, 2831, doi:10.3390/nu13082831.
37. Roy, S.; Rhim, J.-W. Anthocyanin food colorant and its application in pH-responsive color change indicator films. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 2297–2325, doi:10.1080/10408398.2020.1776211.
38. Mattioli, R.; Francioso, A.; Mosca, L.; Silva, P. Anthocyanins: A Comprehensive Review of Their Chemical Properties and Health Effects on Cardiovascular and Neurodegenerative Diseases. *Molecules* **2020**, *25*, 3809, doi:10.3390/molecules25173809.
39. Enaru, B.; Drețcanu, G.; Pop, T.D.; Stănilă, A.; Diaconeasa, Z. Anthocyanins: Factors Affecting Their Stability and Degradation. *Antioxidants* **2021**, *10*, 1967, doi:10.3390/antiox10121967.
40. Oancea, S. A Review of the Current Knowledge of Thermal Stability of Anthocyanins and Approaches to Their Stabilization to Heat. *Antioxidants* **2021**, *10*, 1337, doi:10.3390/antiox10091337.