Reviewer Nurhamidah

|  |  |  |
| --- | --- | --- |
| Thank you very much for valuable comment | |  |
| 1 | *“The sensor displayed an excellent response after 10 minutes of exposure, possessing a response stability for 10 consecutive days. The decrease in pH value of the Tilapia fish from 7.3 to 5 was observed in a 48 hour test, which can be used as the parameter when monitoring fish freshness.“*  Comment: The statement of decrease in pH value need to be elaborated to highlight the novelty of this research. The authors may add comparison in terms of performance and mechanism that differentiate this works and other reported work. E.g How pH value decrease mechanism is evaluated and correlated to observe the fish freshness. | The pH of fresh tilapia was 7.3, and the pH decreased to 5 after 7 hours of storage in two storage conditions (room temperature and 4oC). Changes in pH from 7.3 to 8.7 are the condition of fish monitoring within 48 hours. The post mortem glycolysis-derived lactic acid accumulation is also responsible for the pH decrease.  Additionally we have amended the manuscript with the following text:  “*Our method of measuring the change of pH is different to the most reported studies using colorimetric response. Indeed, one may argue that colorimetry could give the best practicality of the sensor use. However, it suffers from quantitative information, as it depends on the RGB profiles that requires complex model to convert the response into measured pH value. Moreover, the reported studies rely on the volatile basic compounds released from the meat. Taken altogether, the reported studies were unable to capture the decrease of pH during rigor mortis phase. In food industry, fish meat is best processed by the filleting machine during the pre- or post-rigor mortem. This is the novelty of our optical pH sensor which is useful for the quality control and processing of fish meat in industrial settings.*” |
| 2 | **Introduction** Para 2: *"Nevertheless, these aforementioned pH sensors could only be used on solutions with near-neutral pH as more basic or acidic solutions will give an insignificant response time. Pourjavaher et al.11 has designed an optical pH sensor based on cellulose nanofibers with red cabbage (Brassica oleracea) extract, while Rajan et al. (2018)12 has produced an optical pH sensor using peonidin pigment. However, this study did not report the working pH range of peonidin. The use of anthocyanin (ACN) from blackberries and chitosan membrane in an optical pH sensor has been established.13 The interaction and mechanical properties of chitosan membrane with entrapped ACN have also been reported."*  Comment: This paragraph should elaborate more on fish freshness and its correlation to pH based on previous study. The use of ACN should be illustrated for reader to understand more as the sentences is hanging (Referring to *“The interaction and mechanical properties of chitosan membrane with entrapped ACN have also been reported.”*). It is more helpful if a table or illustrated mechanism is shown to support this study and having a good flow of this paper. | The paragraph 2 has been elaborated  *Nevertheless, these aforementioned pH sensors could only be used on solutions with near-neutral pH as more basic or acidic solutions will give an insignificant response time. Pourjavaher et al. 11 has designed a pH sensor using bacterial cellulose (BC) nanofiber matrix to immobilize anthocyanin (CAN) from red cabbage (Brassica oleracea) extract. The sensor has a fairly wide pH range but it needs further characterization to evaluate the sensor performance, especially, for real foodstuff analysis. The use of ACN from blackberries and chitosan membrane in an optical pH sensor has been established. 13 The interaction and mechanical properties of chitosan membrane with entrapped ACN have also been reported. 14 Anthocyanins are flavonoids possessing a number of hydroxyl groups contributing a strong interaction with chitosan via hydrogen bonding.* |
| 3 | Para 3: *"A more recent study on fish freshness monitoring through optical methods was reported by Moradi et al.15 using nanofiber bacterial cellulose with ACN. However, this method requires a relatively long analytical time as the pH measurement could not be conducted in situ. Chen et al. (2020)6 has developed a sensitive novel film prepared from starch polyvinyl alcohol and starch polyvinyl alcohol glycerol."*  Comment: Again, this paragraph does not add the value on published work with this work. The mechanism on optical pH to monitor fish freshness is still not addressed. No comparison on the electrochemical performance (LOD, Linear range, selectivity) was mentioned here. How long analytical time is related to pH measurement by having different material like nanofiber and optical properties coming from ACN dye. The ACN sensitivities towards pH correlation to ACN optical properties may need to be added here as well. | The paragraph 3 has been elaborated line 72-79  *A more recent study on fish freshness monitoring through optical methods was reported by Moradi et al. 15 using nanofiber bacterial cellulose with ACN. However, this method requires a relatively long analytical time as the pH measurement could not be conducted in situ. Chen et al. (2020) 6 has developed a sensitive novel film prepared from starch polyvinyl alcohol and starch polyvinyl alcohol glycerol. The study used curcumin from turmeric and anthocyanin from purple sweet potatoes. The results showed that the mixture of curcumin and ACN improved the stability than that of the individual active substances. As the consequence, the sensor could be employed to detect volatile ammonia as the fish freshness indicator.* |
| 4 | **Research and Methodology**  Comment: The methodology shows a sufficient description a to give reader a good understanding on how this study is conducted. It is suggested that the authors may add process flow/illustration to complete the overall picture on steps and its mechanism. | The steps have been added Methods*Study Design**The first step in sensor fabrication was the extraction of anthocyanin from Ruellia tuberosa L. The extracted anthocyanins were then mixed with pectin solution and printed proportionally as an optical pH sensor. The optical pH sensor was then characterized and the optimized and then applied to monitor the freshness of tilapia. The image below is a schematic diagram summarizing research procedures conducted in this work.* |
| 5 | **Results and Discussion**  Figure 4. SEM profile of (a) PC and (b) ACN/PC membranes.  Comments: The morphology of ACN/PC membrane does not seem like a crack. It seems to have a wavy layer of membrane that might be the contributed to the adhesion/stress tension or air gap of the ACN/PC compared to PC alone. Is there any study on different ration of CAN added to this PC, or is it already optimized? The caption should be more detailed. | The ratio of ACN has been optimized based on the sensitivity and R2, see Table 1 and Figure 8. The description for SEM images analysis has been revised per suggestion. |
| 6 | *"Color change of ACN can be affected by several factors such as temperature, pH, light intensity, sugar moiety and different phenolic derivatives. Due to its solubility in aqueous solution, the color change of ACN is caused by structural transformations of carbon skeleton affected by the levels of H+."*  Comments: The color change mechanism is important to be introduced earlier in the introduction section and can be help with illustration. How different phenolic derivatives change this CAN, and which phenolic derivatives took place in this reaction? The authors may put or add this point to support the color change mechanism towards fish freshness from the finding. | The anthocyanin structure under different pHs has been added in the manuscript as suggested (see Figure 7). |
| 7 | **Effect of PC weight towards sensor sensitivity**  Comments: The pectin is a membrane that hold the ACN dye to improve the sensitivities. From Fig 4, the importance of having optimum load/weight of pectin is important the membrane with less surface tension, and this is the reason of having crack or wavy like membrane. It is very important optimum ratio of CAN/PC to have smooth ACN/PC membrane in this study. | We have optimized the PC weight and the optimum was reached for 0.1% PC to find optimum sensitivity. The membrane with 0.1% w/v pectin has a flatter surface thus making it as the most suitable optical sensor. SEM characterization was carried out on the optimum pectin weight. The wavy like surface structure was probably due to the addition of anthocyanin.  Added as recommendation in conclusion:  *More studies indeed need carried out to obtain smooth surface morphology to improve the optical sensor performance.* |