Potency of Purple Sweet Potato's Anthocyanin as Biosensor for Detection of Chemicals in Food Products

A Wulandari[1], TC Sunarti[1], F Fahma[1] and E Noor[1]

[1] Study Program of Agroindustrial Technology, Department of Agroindustrial Technology, Bogor Agricultural University, Indonesia

Email: titi-cs@apps.ipb.ac.id

Abstract. Bioactive compounds such as anthocyanin are a natural ingredient that produces color with typical specificity. Anthocyanin from *Ayamurasaki* purple sweet potato (*Ipomoea batatas* L.) was extracted in ethanol and used as crude anthocyanin extracts. The color of bioactive anthocyanin can be used as a biosensor to detect chemical of food products because it provides a unique color change. However, the each bioactive has a particular sensitivity and selectivity to a specific chemical, so it is necessary to select and test the selectivity. Six chemicals, which were sodium nitrite, sodium benzoate, sodium cyclamate (food additives), formalin, borax (illegal food preservatives), and residue fertilizer (urea) were tested and observed for its color change. The results showed that the bioactive anthocyanin of purple sweet potato with the concentration of ± 42.65 ppm had better selectivity and sensitivity to sodium nitrite with a detection limit of 100 ppm, where the color change response time ranged from 15-20 minutes. The selectivity and sensitivity of this bioactive can be used as the basic information for the development of biosensor.

1. Introduction

Food safety is an absolute must for foods that are consumed by a human to survive. Mostly found in the excessive amount of chemicals in Indonesian food products were food additives such as sodium benzoate, sodium nitrite, sodium cyclamate, illegal preservatives such as borax and formalin [1], and residue fertilizer as nitrate [2]. Sodium benzoate and sodium nitrite were used for inhibiting the growth of microorganisms like bacteria, mold, and insects [3], sodium cyclamate was mostly used as an artificial sweetener with 30-50 times degree of sweetness compared to sugar [4]. The residue from urea fertilizer was found mainly in the products of fruits and vegetables as nitrate [2]. Those chemicals accumulated in the human body might result in various effects, from cancer, asthma, allergy, skin rash, vasodilator effect, and the generation of carcinogenic nitrosamine and methemoglobin [4-6]. Formalin and borax are highly toxic and carcinogenic chemicals that are not allowed by the government to be used in food products [7, 8]. Thus, it is necessary to search for the way to detect those chemicals. General methods to identify the presence of those chemicals are spectroscopy, spectrophotometry, chromatography like gas chromatography (GC), High-Performance Liquid Chromatography (HPLC), and electrochemical methods [9-12]. However, these methods had disadvantages of requiring high technology and specific experts to operate, restricted to lab-scale, and being multi-step and thus having a long time process [12-14] making it unreliable for routine and economical analysis. Owing to those facts, it is necessary to develop a practical, simple and low-cost chemical sensor for food products with appropriate sensitivity, stability, and selectivity.

The biosensor is a useful device for analyzing bio-molecular interaction in the clinical, biochemical and environmental field. It has the advantages of portable, affordable, easy to use and selective and thus it is widely utilized in food industries to detect harmful contaminants such as metals, pesticide, mycotoxin or pathogenic organisms [15]. One of the biosensors is colorimetric, which works based on color change

In this research, the biosensor for chemicals in food products was developed based on anthocyanin from purple sweet potato. This bioactive is a natural compound which generates colors with particular specificity. Anthocyanin is a bioactive in flavonoid group which has varying color from red to blue and purple and is found in tubers, fruits, flowers, and vegetables with color intensity based on its pH [16-18]. Anthocyanin is water-soluble and is safe to be consumed. Thus it is often used as natural coloring agent [19]. The pigment of anthocyanin is degradable by the exposure of light, pH, temperature, oxygen, ascorbic acid and sugar [20]. Anthocyanin is composed of anthocyanidin components consisting pelargonidin, peonidin, malvidin, petunidin, delphinidin and cyanidin [21].The components in each plant differ depending on its type [22, 23] while the main components of anthocyanin in the purple sweet potato were cyanidin and peonidin [22].

The anthocyanin was extracted from *Ayamurasaki* purple sweet potato (*Ipomoea batatas* L.) in ethanol and was used as the crude anthocyanin extract. The color of this bioactive can be utilized as the biosensor to detect chemical in food products because the color changes significantly when interacting with a particular chemical compound. However, anthocyanin has a particular sensitivity and selectivity to each chemical. Therefore, it is required to conduct tests to investigate the sensitivity and selectivity to particular chemicals. This research aims to investigate the selectivity and sensitivity of anthocyanin against various chemicals in food products (sodium benzoate, sodium nitrite, sodium cyclamate, urea fertilizer, formalin, and borax).

2. Materials and methods

2.1. Materials

The materials in this work were *Ayamurasaki* purple sweet potatoes (*Ipomoea batatas* L.) which were obtained in the local market in Cikarawang, Bogor. Acetic acid, formalin, borax (sodium tetraborate) and urea were from Merck (Darmstadt, Germany); distilled water, ethanol 96%, sodium benzoate, sodium cyclamate were obtained from "*Setia Guna*" chemical shop in Bogor. Sodium nitrite was from "*Brataco"* chemical shop in Bogor, Indonesia. The blender (Philips), centrifuge (IEC Clinical Centrifuge, USA), vacuum filter (Value, 2 Stage Vacuum Pump, VE 2100 N), filter paper, water bath, pH meter (Beckman), spectrophotometer UV-VIS (Thermo Scientific, Genesys 10S, Germany), digital camera (Canon, Power Shot A2300 HD) and glass vessels were used for analysis.

2.2. Sample preparation and anthocyanin extraction from purple sweet potato

The purple sweet potato was washed, peeled, and cut with ± 3 cm length. The sample then was blanched for 7 minutes, mashed and frozen at -27^{o}C. The frozen sample with 350 g was crushed with blender for 3 minutes with an addition of 700 mL solvent (solvent: sample = 1:2). The solvent consisted of ethanol 96%, acetic acid, and distilled water with a ratio of 25:1:5, respectively. Next, the extract was drained with fabric. The filtrate was heated in water bath at 50 ^{o}C to vaporize ethanol to obtain a more concentrated filtrate. The pigment filtrate then was filtered using vacuum filter with filter paper. The filtrate was centrifuged with 3000 rpm for 15 minutes. After that, the supernatant was kept in a dark glass bottle in a refrigerator for further analysis.

2.3. pH measurement of chemicals

Chemicals (sodium cyclamate, sodium benzoate, sodium nitrite, urea, formalin, and borax) was prepared each as a solution with 10% concentration and was measured by pH meter.

2.4. Selectivity test of purple sweet potato's anthocyanin against chemicals

Anthocyanin has a particular sensitivity and selectivity to specific chemicals. In this research, anthocyanin was tested for sodium cyclamate, borax, sodium nitrite, urea, formalin, and borax. This test aimed to investigate the chemicals which elicited a significant color change after the reaction with anthocyanin, thus providing vital information for further development of biosensor.

Crude anthocyanin extract with a concentration of 42.65 ppm was added with each chemical solution (sodium cyclamate, sodium benzoate, sodium nitrite, urea, formalin, and borax) in a high concentration of 1% and 10%, with a ratio of 1:4 (anthocyanin: chemical). After 15 minutes, the color change was observed, and the absorbance was measured using spectrophotometer UV-VIS in the range of 400-760 nm.

2.5. Sensitivity test of purple sweet potato's anthocyanin to selected chemicals

Chemicals from previous test which reacted to anthocyanin underwent further analysis with broader range of concentration: 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, and 10,000 ppm, by reacting anthocyanin solution (42.65 ppm) with each selected chemical in the concentration of 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, and 10,000 ppm, with a ratio of 1:4 (anthocyanin: selected chemical). The color change was recorded with a digital camera for ± 30 minutes, and the absorbance was measured with the spectrophotometer.

3. Results and discussion

3.1. pH measurement of chemicals

Table 1 shows the result of pH measurement of chemicals. Sodium nitrite, sodium benzoate, urea, and borax had a pH ranging from 7 – 9, whereas sodium cyclamate tended to be neutral and formalin was acidic. This characterization was conducted to identify the sensitivity of anthocyanin to the chemicals with a particular pH value with a color change from red to purple, blue, green and yellow, which was also reported in [24]. Anthocyanin colored red at pH of 1 – 2, blue at pH of 6.5 – 9, and yellow at pH > 9 [24]. With these results, we can predict the chemical color change when reacting with anthocyanin and then be matched against the observed results of the anthocyanin's sensitivity to the chemicals.

Table 1. pH of chemicals

Chemicals	pH
Sodium cyclamate	6.532
Sodium nitrite	8.986
Sodium benzoate	7.802
Urea	8.052
Borax	9.342
Formalin	3.004

3.2. Selectivity of anthocyanin purple sweet potato's against chemicals

3.2.1. Color characteristics of purple sweet potato's anthocyanin to chemicals. Figure 1 displays the result of the test where it can be seen that anthocyanin has a good selectivity against sodium nitrite to the concentration of 1% (10,000 ppm), indicated by the significant color change from reddish to yellowish. The color change into yellowish was owing to the nucleophilic substitution of –nucleophiles (nitrate ion) at the C-4 position in the flavylium structure of anthocyanin and thus forming a complex of anthocyanin-nitrite.

Figure 1. Purple sweet potato's anthocyanin selectivity to chemicals(1%) (KL: control/anthocyanin, NS: sodium cyclamate, NB: sodium benzoate, NN: sodium nitrite, UR: urea, FN: formalin, BX: borax.

The formation of anthocyanin-nitrite induced flavylium cation (red) to transform into chalcone (yellow) [25, 26]. Meanwhile, other chemicals (sodium benzoate, cyclamate, urea, formalin, and borax) did not react well with anthocyanin as there was no significant change in color; they were relatively stable at reddish color. Based on pH measurement, sodium nitrite had a pH of 8.9. Anthocyanin of red sweet potato had a bluish color in pH of 8-9 and had a color change into yellowish when in pH of 11.7 [27]; thus we could say that the change occurred not only due to the pH change, but also a reaction occurred between nitrite ion and flavylium ion in anthocyanin. We concluded that anthocyanin of purple sweet potato had an excellent sensitivity to sodium nitrite.

Flavonoid from *Schisandra chinensis* fruit powder could enhance reddish color in sausage, indicated by the increase of a* (redness) in L*a*b system; however the addition of nitrite as preservative caused sausage color to fade and turned into yellowish, indicated by the increase of b* (yellowness) [28]. This phenomenon showed that nitrite was able to alter the structure of anthocyanin and fade its color into yellowish.

However, in the test of selectivity of anthocyanin to chemicals at 10% concentration each, there was an interaction to borax as well. Figure 2 shows the result of the anthocyanin-borax interaction, resulting in a bluish solution (from reddish).

Figure 2. Purple sweet potato's anthocyanin selectivity against chemicals (10%) (KL: control/anthocyanin, NS: sodium cyclamate, NB: sodium benzoate, NN: sodium nitrite, UR: urea, FN: formalin, BX: borax.

Anthocyanin of purple sweet potato turned into bluish after reacting with borax (10%), most likely due to flavylium ion which gave reddish color turned into quinonoid compound by the presence of borax substituting the position of C-4 [24-26]. Despite that, the chemical contaminant selected for further analysis was sodium nitrite for its better (significant) sensitivity.

3.2.2. Spectrum characterization of purple sweet potato's anthocyanin against chemicals by spectrophotometer UV-VIS in 400-760 nm. Spectrophotometry showed that anthocyanin (KL), sodium cyclamate (NS), benzoate (NB), formalin (FN) and borax (BX) had a peak at 540 nm while urea had a peak at 520 nm. Figure 3 shows the details of spectrophotometry result.

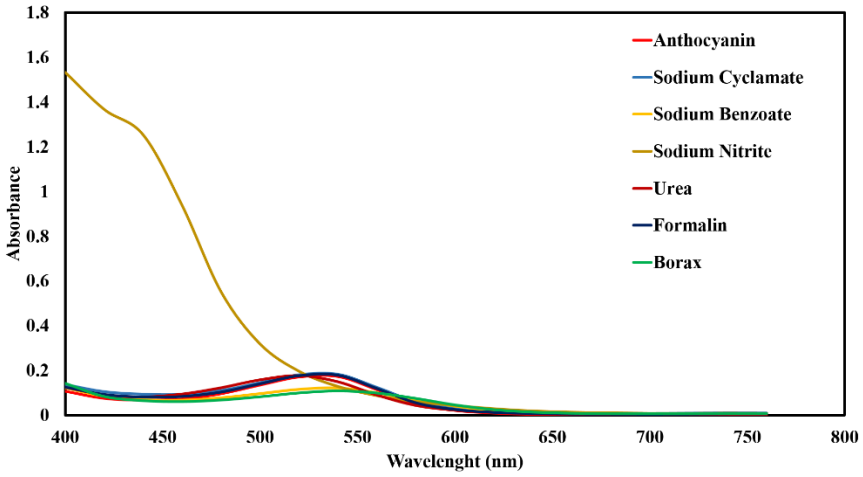

Figure 3. Spectrum of the samples (anthocyanin-1% chemicals) in **λ** 400-760 nm

This result describes insignificant color change of the anthocyanin-chemicals (NS, NB, FN, BX, and UR) reaction, where the colors were stable at reddish color. At the wavelength of 520 and 540 nm, the visible complementary color of red was reflected, showing there was no reaction between purple sweet potato's anthocyanin and NS, NB, FN, BX, and UR. In contrast, the interaction of purple sweet potato's anthocyanin and sodium nitrite (NN) resulted in the peak at the wavelength of 440 nm. There was a hypsochromic shift from 540 nm (anthocyanin) to 440 nm (anthocyanin-nitrite), indicating a color change after anthocyanin reacted with sodium nitrite, which was pinkish-red to yellow.

The absorbance of each solution (anthocyanin-chemicals) at 440 nm clearly explained that only anthocyanin-sodium nitrite had a peak (figure 4), resulting in a color change to yellowish after anthocyanin reacted with sodium nitrite. This is because there is a change of flavylium ions from anthocyanin (red) to form chalcone (yellow) [25, 26]. In a case of lead (Pb) detection by using curcumin, the spectrophotometry result showed the curcumin-lead had a peak at specific wavelength whereas the solution of curcumin and other metal ions had not [14].

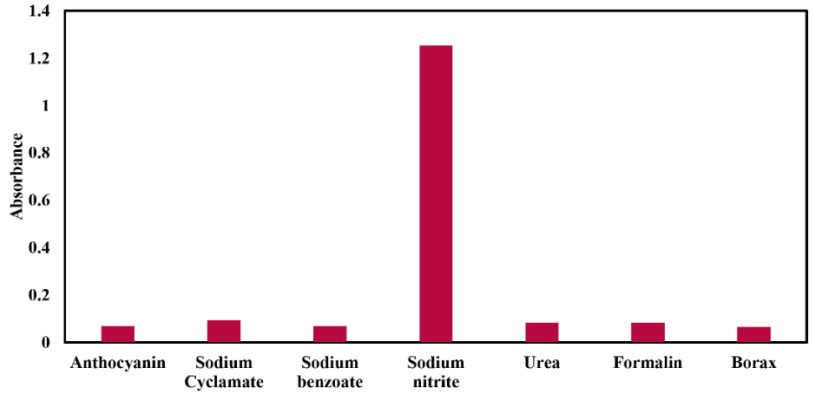

Figure 4. The spectrum of samples (anthocyanin-1% chemicals) at 440 nm

As described in figure 4, we concluded that anthocyanin of purple sweet potato had an excellent selectivity to sodium nitrite and was not influenced by the presence of other chemicals (sodium benzoate, cyclamate, urea, borax, and formalin). Thus, sodium nitrite was selected as the sample for sensitivity test with a concentration range of 1% to 10%.

3.3. Sensitivity test of purple sweet potato's anthocyanin to sodium nitrite

3.3.1. Color characterization of purple sweet potato's anthocyanin against sodium nitrite. The sensitivity test was undergone to anthocyanin against sodium nitrite in the concentration of 10–10,000 ppm. Figure 5 exhibits the result that there is color degradation from pinkish-red color to pale orange to bold yellow (from the addition of 0 – 10,000 ppm of sodium nitrite/NN). In the sample of anthocyanin-sodium nitrite (100 ppm), the color started to change into pale orange. A significant change of color into yellowish was revealed in the sample with 500 ppm of NN.

Figure 5. Color sensitivity of purple sweet potato's anthocyanin against sodium nitrite in various concentrations (ppm)

This result meant that the limit of detection (LOD) of purple sweet potato's anthocyanin to detect the presence of sodium nitrite was 100 ppm with the anthocyanin concentration of ± 42.65 ppm. The duration until the color change differed for each sample with different concentration. The higher the concentration of NN was, the change was faster. The response time rate of anthocyanin to change color after reacting with sodium nitrite was 15 – 20 minutes.

In a test of the colorimetric sensor using nanoparticle of Ag@Au to detect sodium nitrite, it could detect sodium nitrite with a LOD of 1 μM while it had a good selectivity against other ions as well [29]. The sensor showed a change from purplish to reddish with the presence of nitrite [29]. Compared to that case, this research had lower sensitivity, most probably due to the use of nanoparticle in that other research. The use of nanoparticle might enhance the absorbance of nitrite. Nonetheless, this research has the advantage of using an eco-friendly bioactive – which was anthocyanin, and is affordable as well, for the sensor.

3.3.2. Spectrum characterization of purple sweet potato's anthocyanin-sodium nitrite by using spectrophotometer UV-VIS in the wavelength of 400-760 nm. Spectrophotometry result exhibited that the solution of anthocyanin-nitrite in sodium nitrite (NN) concentration of 0-250 ppm had a peak at 540 nm, whereas the solution with an NN concentration of 500-10,000 ppm had a peak at 440 nm, indicating a hypsochromic shift from 540 nm (NN concentration of 0-250 ppm) to 440 nm (NN concentration of 500-10,000 ppm). This phenomenon can be observed in figure 6.

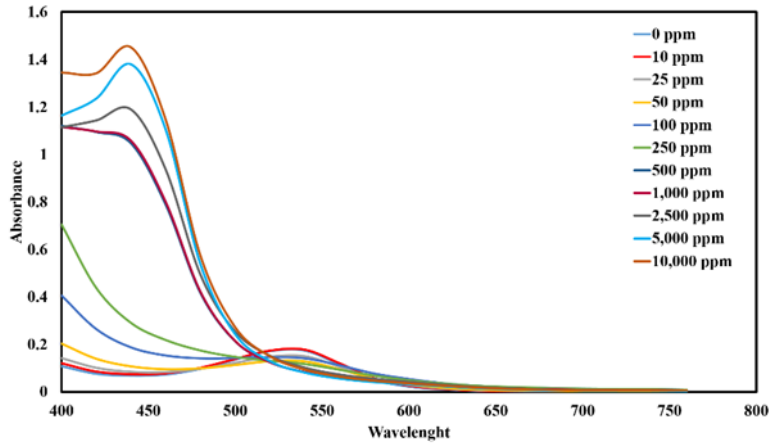

Figure 6. The spectrum of purple sweet potato's anthocyanin against sodium nitrite in various concentrations (0-10,000 ppm), at 400 - 760 nm

The shifting of wavelength indicated there was a color change from pinkish-red to yellowish. Beside hypsochromic shifting, a hyperchromic shift occurred from the solution with sodium nitrite concentration of 0-250 ppm (540 nm) to the solution with 500-10,000 ppm sodium nitrite (440 nm). Hyperchromic, the shifting from lower concentration to a higher concentration, was due to the color change of anthocyanin-nitrite from pinkish-red to bold yellow.

Figure 7 displays the peak drop of anthocyanin-nitrite along with the increase of concentration (from the concentration of 0-250 nm) at the peak of 540 nm. This drop indicated there was a paling of reddish color of anthocyanin at 540 nm. This phenomenon was also reported in the case of peak drop of Ag@AuNP at 526 nm in the solution with various nitrite concentrations of 0.1-100 µM [29] and as well in the case of the curcumin-lead peak at 456 nm in the various lead concentration of 10-100 µM [14].

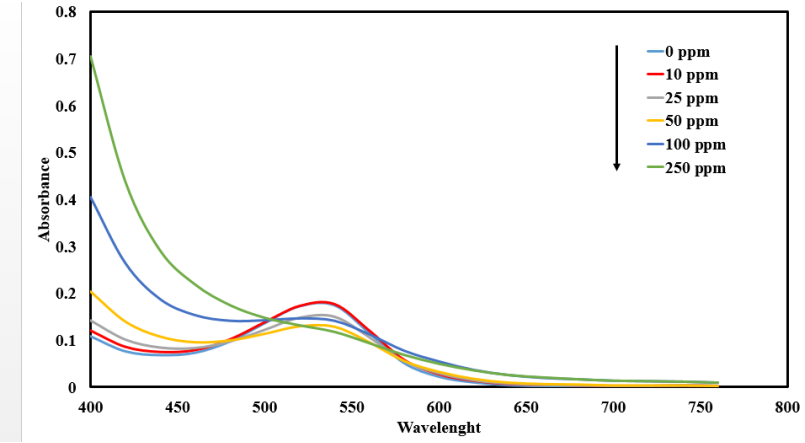

Figure 7. The spectrum of purple sweet potato's anthocyanin against sodium nitrite in various concentrations (0-250 ppm), at 400-760 nm

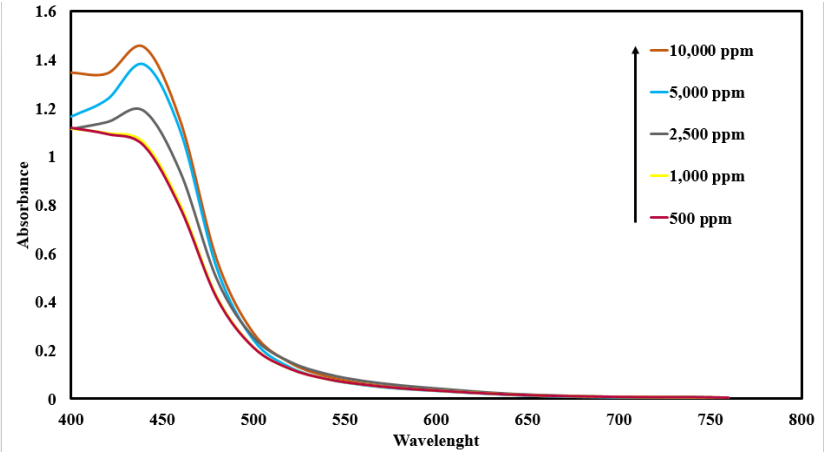

Figure 8. The spectrum of purple sweet potato's anthocyanin against sodium nitrite in various concentrations (500-10,000 ppm), at 400-760 nm

The increase of absorbance to 440 nm (**λ** = 400-760 nm) occurred in the anthocyanin-nitrite solution with various sodium nitrite concentrations of 500-10,000 ppm. The absorbance increased along with the increase of sodium nitrite concentration thus the yellow color intensified. In the case of the sensor to identify formaldehyde using anthocyanin reaction with hydroxylamine sulfate, there was also an increase of spectrum absorbance along with the increase of formaldehyde concentration (0.0-6.4 ppm) at 506 nm [30].

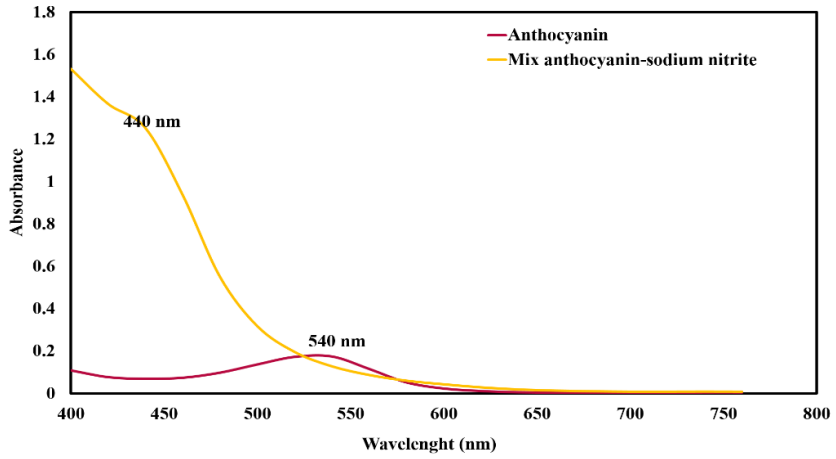

Figure 9. The spectrum of anthocyanin (control) and anthocyanin-nitrite (10,000 ppm) at 400-760 nm

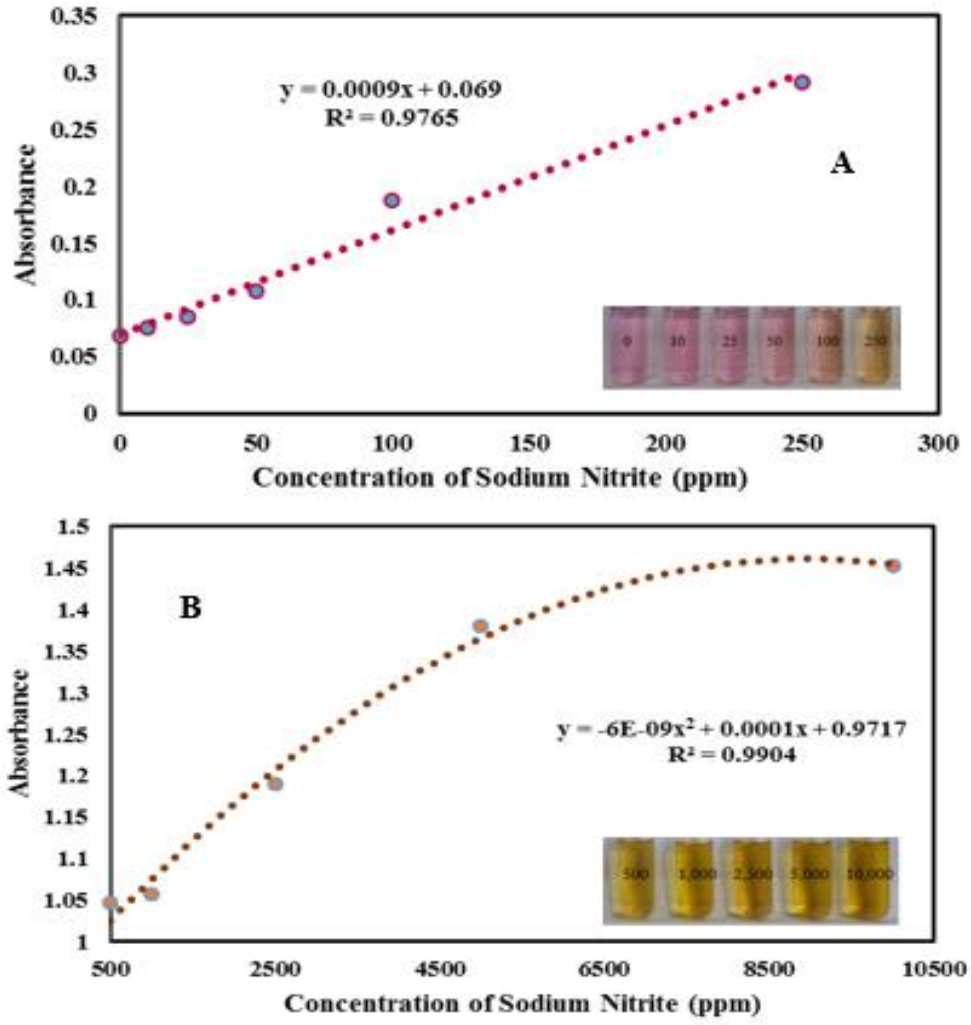

Figure 10. The calibration plot of absorbance of anthocyanin-nitrite at 440 nm and nitrite concentration range of 0-250 ppm (Fig. 10A) and 500-10000 ppm (Fig. 10B).

Figure 9 shows the comparison of the absorbance of anthocyanin and anthocyanin-nitrite. Anthocyanin had a peak at 540 nm while anthocyanin-nitrite had the peak at 440 nm, explaining that the color of the reddish color of anthocyanin changed to the yellowish color of anthocyanin-nitrite after anthocyanin reacted with sodium nitrite.

3.4. Analytical performance

Figure 10 was a plot calibration between absorbance of anthocyanin-nitrite at 440 nm and nitrite concentration range of 0-250 ppm (Fig. 10A) and 500-10000 ppm (Fig. 10B). The relationship of both produced a linear equation with the value of the equation of y= 0.0009x + 0.069 and the correlation coefficient of 0.9765 (Fig.10A) showing that there was an increase in absorbance as the concentration of sodium nitrite increases. Figure 10B shows the correlation curve between the absorbance of anthocyanin-nitrite and the concentration of nitrite form a polynomial line with a correlation coefficient of 0.9904, indicating that an increase in absorbance as the sodium nitrite concentration increases to the optimum point until constant.

4. Conclusions

Purple sweet potato's anthocyanin of ± 42.65 ppm had excellent sensitivity and selectivity to sodium nitrite. Anthocyanin could detect sodium nitrite with a limit of detection of 100 ppm. The color change response time of the reaction of anthocyanin with sodium nitrite ranged from 15 to 20 minutes. These properties of selectivity and sensitivity were the primary considerations of the development of biosensor. Hence, anthocyanin extracted from *Ayamurasaki* purple sweet potatoes has a great potential to be implemented into an eco-friendly, easy to use, affordable, and has a proper sensitivity and selectivity biosensor development for sodium nitrite detection.

Acknowledgement

This research especially thank the Direktorat Jenderal Penguatan Riset dan Pengembangan Kementerian Riset, Teknologi dan Pendidikan Tinggi Lembaga Penelitian dan Pengabdian Kepada Masyarakat, Indonesian Government for the funding and support to this research, with is stated at 1105/IT3.11/LT/2017.

References

[1] National Agency for Drug and Food Control 2016 Annual report of result of security and quality supervision food products [in Indonesian]. http://www.pom.go.id/new/admin/dat/20171127/laptah2016.pdf [2018-01-07]
[2] Cintya H, Silalahi J, Putra E D L and Satria D 2016 Analysis of Nitrite and Nitrate in Vegetables in Medan City *Der. Pharma. Chemica* **8** 47-52
[3] Sharma S 2015 Food Preservatives and their harmful effects *IJSRP* **5** 1-2
[4] Pandey RM and Upadhyay SK 2012 *Food Additive* ed Yehia El-Samragy (Europe : *InTech*) chapter 1 pp 1-30 http://www.intechopen.com/books/food-additive/food-additive [2017-05-05]
[5] Mitacek E J, Brunnemann K D, Suttajit M, Caplan LS, Gagna C E, Bhothisuwan K, Siriamornpun S, Hummel CF, Ohshima H, Roy R and Martin N 2008 Geographic distribution of liver and stomach cancers in Thailand in relation to estimated dietary intake of nitrate, nitrite, and nitrosodimethylamine *Nutr. Cancer* **60** 196–203
[6] Marco A, Navarro J L and Flores M 2006 The influence of nitrite and nitrate on microbial, chemical and sensory parameters of slow dry fermented sausage *Meat. Sci*. **73** 660–673
[7] Dengbin Yu , Daming Yong and Shaojun Dong 2013 Toxicity detection of sodium nitrite, borax and aluminum potassium sulfate using electrochemical method *J Environ Sci.* **25** 785-90
[8] IARC (International Agency for Research on Cancer) 2006 Monographs on the evaluation of carcinogenic risks to humans, vol. 88. Formaldehyde, 2-butoxyethanol and 1-tert-butoxy-2-propanol http://inchem.org/documents/iarc/vol88/volume88.pdf [2017-08-19]

[9] Tang J H, Wang X M, Sheng G Y and Fu J M 2005 The analytical research progress of formaldehyde and carbonyl compound in air *Analytical Chemistry* **33** 134-140 (in Chinese)
[10] Davis J, Compton R G 2000 Sonoelectrochemically enhanced nitrite detection *Analytica Chimica Acta*. **404** 241-7
[11] Davis J, Moorcroft M J, Wilkins SJ, Compton R G and Cardosi M F 2000 Electrochemical detection of nitrate and nitrite at a copper modified electrode *Analyst*. **125** 737-742
[12] Chiou J, Arthur H H L, Hang W L and Wing-tak W 2015 Rapid testing methods for food contaminants and toxicants *Journal of Integrative Agriculture* **14** 2243-64
[13] Zhao Z and Cai X 1988 Determination of trace nitrites by catalytic polarography in ferrous iron thiocyanate medium *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* **252** 361-370
[14] Raj S and Dhesingh RS 2016 Curcumin based biocompatible nanofibers for lead ion detection *Sensors and Actuators* B **226** 318-25
[15] Santoro K and Ricciardi C 2016 *Biosensors*. Politecnico di Torino– LATEMAR Unit (Torino, Italy)
[16] Torskangerpoll K and Andersen OM 2005 Colour stability of anthocyanins in aqueous solutions at various pH values *Food Chem.* **89** 427-40
[17] Burdulis D, Sarkinas A, Jasutiené I, Stackevicené E, Nikolajevas L and Janulis V 2009 Comparative study of anthocyanin composition, antimicrobial and antioxidant activity in bilberry (*Vaccinium myrtillus* L.) and blueberry (*Vaccinium corymbosum* L.) fruits. *Acta Pol.Pharm.* **66** 399-408
[18] Jensen M B, Bergamo C A, Payet R M, Liu X and Konczak I 2011 Influence of copigment derived from Tasmannia pepper leaf on Davidson's plum anthocyanins *J.Food.Sci*. **76** C447–53
[19] Chisté R C, Lopes A S and Faria L J G 2010 Thermal and light degradation kinetics of anthocyanin extracts from mangosteen peel (*Garcinia mangostana* L.) *Int.J.Food.Sci Tech*. **45** 1902-08
[20] Castañeda-Ovando A, Pacheco-Hernández M L, Páez-Hernández M E, Rodriguez J A and Galán-Vidal CA 2009 Chemical studies of anthocyanins: A review *Food Chem.* **113** 859-871
[21] Harborne JB 1967 *Comparative biochemistry of the flavonoids* (New York:Academic Press)
[22] Montilla EC, Hillebrand S, Butschbach D, Baldermann S, Watanabe N and Winterhalter P 2010 Preparative isolation of anthocyanins from Japanese purple sweet potato (*Ipomoea batatas* L.) varieties by high-speed countercurrent chromatography *J. Agric. Food Chem.* **58** 9899-9904
[23] Terahara N, Saito N, Honda T, Toki K and Osajima Y 1990 Structure of ternatin A1, the largest ternatin in the major blue anthocyanins from *Clitoria ternatea* flowers *Tetrahedron Lett*. **31** 2921-24
[24] Março P H, Poppi R J, Scarminio I S and Tauler R 2011 Investigation of the pH effect and UV radiation on kinetic degradation of anthocyanin mixtures extracted from *Hibiscus acetosella Food Chem*. **125** 1020-27
[25] Fichtner C Grigoriy R and Herbert M 2001 Kinetics of the Reactions of Flavylium Ions with π-Nucleophiles *Eur. J. Org. Chem.* **23** 4451-56
[26] Galán-Vidal C A, Castañeda-Ovando A, Páez-Hernández M E and Contreras-López E 2014 Determination of Nitritees in Commercial Sausages by Anthocyanins Degradation. Experimental Design and Optimization *J. Mex. Chem. Soc.* **58** 180-184
[27] Cevallos-Casals B A and Cisneros-Zevallos L 2004 Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants *Food Chem*. **86** 69–77
[28] Jin S K and Park J H 2013 Effect of the addition of *Schisandra chinensis* powder on the physico-chemical characteristics of sausage *Asian-Australas.J.Anim.Sci.* **26** 1753-61
[29] Li T, Li Y, Zhang Y, Dong C, Shen Z and Wu A 2015 A colorimetric nitritee detection system with excellent selectivity and high sensitivity based on Ag@Au nanoparticles *Analyst* **140** 1076-81

[30] Meng Q, Han T , Wang G , Zheng N , Cao C and Xie S 2014 Preparation of a natural dye doped Ormosil coating for the detection of formaldehyde in the optical gas sensor *Sensors and Actuators* B **196** 238-244