The potential of bioactives as biosensors for detection of pH

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Abstract. In the field of food safety, pH measurement is very important because pH can be an indicator of freshness of a food product. In general, the pH measurement is performed using a sensor from synthetic chemicals. The use of chemicals as pH sensors has several disadvantages that are toxic and threaten human health. Therefore, pH biosensors should be developed from bioactive compound. Bioactives such as curcumin, anthocyanin, chlorophyll, and Angkak pigments are indigenous natural resources that produce color with specific specificity. Curcumin was extracted from turmeric (Curcuma longa L.), anthocyanin from purple sweet potato (Ipomoea batatas L), chlorophyll from suji leaf (Pleomele angustifolia), and reddish pigment from rice fermented by Monascus sp. The type of color of each bioactive can give different responses to certain pH so that it is potential to be developed as a pH biosensor. However, bioactives have sensitivity to certain pH, so it is necessary to test the sensitivity and selection of bioactives to various pH (2-13). The results of the sensitivity of the bioactives to pH 2-13 showed that anthocyanin and curcumin were the best bioactive as pH biosensor because they provide a unique and significant color change compared to angkak pigment and chlorophyll which did not show clear discoloration. The anthocyanin was sensitive to pH 5-13 and curcumin was sensitive to pH 7-13 and both were stable at acidic pH.

Keywords : bioactive, color change, biosensor, pH

1. Introduction

In the field of food safety, pH measurement is very important because pH can be an indicator of freshness of a food product. In general, the pH measurement is performed using a sensor from synthetic chemicals. The use of chemicals as pH sensors has several disadvantages that are toxic and threaten human health. Therefore, it is necessary to develop the pH detector sensor quickly, simple, cheap, easy to use and not toxic. Colorimetric sensor is a method often used for pH detection is easy to use, inexpensive, and on-site application [1]. Biosensor as an innovative device for analysis offers advantages such as selective, affordable, portable and easy to use; thus it is widely used in food industries to detect food contaminants like metals, pathogenic microorganism, mycotoxin, antibiotics
residue, and pesticide [2]. Several biosensors work under the change of color to detect dangerous chemical contaminant, known as colorimetric.

The diversity of natural plant resources is a good source for colorant and bioactive compounds that will offer an opportunity to be a biosensor with a unique specificity and sensitivity. In this study, biosensors for pH measurements of food products were developed from the bioactives such as anthocyanins from purple sweet potato (*Ipomoea batatas* L), curcumin from turmeric (*Curcuma longa* L), reddish pigment from red rice fermented by *Monascus* sp and chlorophyll from suji leaf (*Pleomele angustifolia* NE Brown).

Curcumin is a natural yellowish-orange dye from turmeric (*Curcuma longa* (L)) or other curcuma compounds[3-5]. Anthocyanins are a bioactive compound from flavonoid group with various color range, from purple, red, and blue, found in the tubers, leaves, fruits, flowers, and vegetables with color intensity depends on its pH [6-8]. Chlorophyll is the main pigment that gives a green color to a plant that has a central atom of Mg$^{2+}$ [9]. Angkak is rice fermented by *Monascus purpureus* mold so that the appearance is red [10]. However, bioactives have sensitivity to certain pH, so it is necessary to test the sensitivity and selection of bioactives to various pH. Therefore, the purpose of this study was to select the best bioactives as pH detecting biosensors by analyzing their sensitivity to various pH. To our knowledge so far, pH sensitivity examination from acidic to alkaline pH against various bioactives from four different natural resources has not been widely reported by previous studies, so this study provides new information in the future as a basis for biosensor development of pH sensing.

2. Materials and Methods

2.1. Materials

Turmeric (*Curcuma longa*), purple sweet potato varieties of *Ayamurasaki*, suji leaf (*Pleomele angustifolia*), Angkak, ethanol, Sodium bicarbonate (NaHCO$_3$), acetic acid, citric acid monohydrate 0.1 M (C$_6$H$_5$O$_7$•H$_2$O), trisodium citrate 0.1 M (C$_6$H$_5$O$_7$•Na$_3$•2H$_2$O), sodium phosphate dibasic dihydrate (Na$_3$HPO$_4$•2H$_2$O) 0.2 M, sodium phosphate monobasic, (NaH$_2$PO$_4$•H$_2$O) 0.2 M, potassium chloride (KCl), hydrochloric acid (HCl), sodium hydroxide (NaOH), and phosphoric acid were purchased from Merck (Darmstadt, Germany). Ethanol 96%, distilled water, and technical grade of Tween 80 were also used in this study. Equipment in this research included UV-VIS spectrophotometer (Thermo Scientific, Genesyss 10S), Liquid Chromatography Mass Spectrometry / LC-MS (Waters Alliance 2695), High-Performance Liquid Chromatography / HPLC (Hitachi), vacuum filter (Value, 2 Stage Vacuum Pump, VE 2100 N), shaker (Banstread 2346-ICE), Whatman filter paper No. 41, filter paper, analytical scales (Sartorius BL 2105), centrifuge (IEC Clinical Centrifuge, USA), water bath and magnetic stirrer (Lab tech Multi-Position), pH meter (Beckman), blender, oven blower (Memmert), and glassware used for analysis.

2.2. Preparation and extraction of bioactives

The anthocyanin extraction of purple sweet potato was adopted from Mahmudatussa'adah [11] and Winarti et al. [12] methods. Curcumin was extracted from turmeric that adopted the methods of Setyowati and Suryani [13] and Waghmare [14]. Extraction of chlorophyll was adopted from Prangdimurti [15] and red pigment extraction from Angkak was from Suh and Shin [16] and Tismanjaja and Irawan [17].

2.3. Preparation of Buffer solution

The buffer solutions of pH 2-3 were prepared from mixture of KCl-HCl (0.2 M, respectively). The pH 4-6 buffer solutions were prepared from mixture of citric acid monohydrate and trisodium citrate dihydrate (0.1 M, respectively). Buffer solutions of pH 7-9 were prepared from mixture of sodium phosphate (0.2 M respectively). Lastly, buffer of pH 10-13 were prepared from mixture of KCl-NaOH (0.2 M respectively).
2.4. Bioactives Characterization

2.4.1. Measurement of bioactives concentrations

The measurement of the concentration of anthocyanin extracts was adopted from methods of Cheng and Breen [18] and Giusti and Wrolstad [19]. Two sample solutions were prepared, the first solution was buffer solution of pH 1.0 (KCl buffer) and the second solution was buffer solution of pH 4.5 (sodium acetate buffer). Furthermore, each purple sweet potato anthocyanin extract was taken as much as 1 mL and then diluted using each the buffer solution to a volume of 10 mL (dilution factor = 10). The dilution samples were measured for absorbance at λ 510 and λ 700 nm, respectively [18]. The measurement method was adopted from Giusti and Wrolstad [19] using the following equation.

\[
A = (A_{510} - A_{700}) \text{pH 1.0} - (A_{510} - A_{700}) \text{pH 4.5} \quad \text{.................................. (1)}
\]

With total anthocyanin concentration was calculated based on the following equation 2:

\[
\text{Total of anthocyanin (mg/L)} = (A \times Df \times MW \times 1000) / (\varepsilon \times 1) \quad \text{........................................ (2)},
\]

with Df: dilution factor, A: absorbance value, MW: molecular weight (449.2), \(\varepsilon\): molar absorptivity coefficient (26900).

Measurement of curcumin concentration was using the method adopted from Holkar et al. [20] and Harini et al. [21]. Curcumin standard curves were prepared. From the standard curve, the equation \(y = ax + b\) was obtained. Based on the equation, curcumin concentration was calculated based on equation 3.

\[
x = (y - b) / a \quad \text{.................................................. (3)},
\]

\(x = \) concentration of curcumin, \(y = \) absorbance value, \(a = \) coefficient of optical density (OD) value, \(b = \) constant of optical density (OD) value.

The concentration of chlorophyll extract was measured using the method of Gross [9]. About of 1.5 mL chlorophyll extract was mixed with 8.5 mL of 99.5% acetone. Then left it for one night in the refrigerator. Next the solution was centrifuged at 600 g (3000) rpm for 10 minutes. Furthermore absorbance measurements were carried out with a UV-VIS spectrophotometer at wavelengths of 645 nm and 663 nm or 652 nm. Chlorophyll concentration was calculated based on equation 4.

\[
\text{Total of chlorophyll (mg/L)} = 20.2 A_{645 \text{ nm}} + 8.02 A_{663 \text{ nm}} \quad \text{.......................... (4a)}
\]

or

\[
\text{Total of chlorophyll (mg/L)} = (1000 / 34.5) A_{652 \text{ nm}} \quad \text{.......................... (4b)}
\]

The red pigment concentration of Angkak was measured by the method adopted from Dhale [22]. Concentration of red yeast pigment was determined by measuring it directly using a UV-VIS spectrophotometer at 500 nm. Furthermore the concentration was calculated using OD Unit with the formula in equation 5.

\[
\text{OD Units} = (A \times \text{total of solvent volume} \times \text{dilution factor}) / (\text{Weight of red yeast rice (g)}). \quad \text{(5)}
\]

2.4.2. Characterization of bioactive components

The determination of the components contained in anthocyanin of purple sweet potato, curcuminoid of turmeric, suji leaf chlorophyll, and Angkak pigment was determined using a chromatography column in the form of Liquid Chromatography Mass Spectrometry (LC-MS) [23; 24].

2.5. Bioactive sensitivity testing against various pH 2-13

Sensitivity analysis was performed by measuring the spectra of bioactive extracts in the range of pH 2-13 which refers to Cevallos-Casals and Cisneros-Zevallos [25]. As much as 1 ml of sample was
poured into the test tube and then was added with 4 ml of buffer solution from pH 2-13, respectively, after 15 min, absorption (A) were measured using spectrophotometer (UV- VIS) at \( \lambda = 280-800 \) nm.

3 Results and Discussion

3.1 Concentration of bioactives extract in each natural resource

Each of these bioactive was extracted using ethanol with a simple method that is maceration. Ethanol was the best choice for the extraction of anthocyanins, curcumin, chlorophyll and the red pigment of red yeast rice as it gave the intensity and color brightness which was good and not easily covered by molds. The results of concentration and yield of each bioactive extract in crude ethanolic extract are presented in Table 1.

| Extract                          | Concentration       | Yield            |
|----------------------------------|---------------------|------------------|
| Extract of purple sweet potato pigment | 214.75 ± 0.884 (mg/L) | 0.43 ± 0.0017 mg/g tuber |
| Extract of Turmeric pigment      | 2190.75 ± 1.931 (mg/L) | 15.34 ± 0.013 mg/g dry powder |
| Extract of Suji leaf pigment     | 30.08 ± 0.044(mg/L)  | 0.30 ± 0.0004 mg/g fresh leaf |
| Extract of red pigment of Angkak | 41.625 ± 75(OD.U)   | 416.25 ± 0.75 OD.U/g dry powder |

Note: replication, n=3

Based on Table 1 the concentration of pigment from fresh purple sweet potato (anthocyanin) extracted with ethanol was 214.75 ± 0.884 mg/L filtrate with the yield of 0.43 mg/g fresh tuber of purple sweet potato. Chumsri et al. [26] reported that the anthocyanin content of fresh rosella petals extracted with water was 36.67 ± 0.02 mg/100 g. According to Jiao et al. [27] the total anthocyanin content of purple sweet potato was 1.38 mg/g. The results of Yudio [28] showed that the content of anthocyanin from extract of purple sweet potato with extraction using subcritical water was 0.4298 mg/g. The difference of anthocyanin concentration was extracted due to differences in varieties, places and growing environments [29] as well as the types of sources of raw materials and extraction methods used. The pigment extract of turmeric (curcumin) extracted with ethanol in this study had a concentration of 2190.75 ± 1.931 mg/L filtrate with the yield of 15.34 mg/g turmeric powder or 1.534%. This result is not much different from the research reported by Paulucci et al. [30] which is about 0.1-1.8% (Curcuma longa).

The extract concentration of fresh suji leaf pigment (chlorophyll) obtained in this study was 30.08 ± 0.044 mg/L. These results are not much different from the results of research Aryanti et al. [31] which obtained the concentration of chlorophyll suji leaf of 30.327-41.939 mg/L. The use of solvent volume and type of solvent affects the resulting chlorophyll content.

Angkak red pigment content obtained from this study amounted to 416.25 ± 0.75 OD.U/g angkak dry powder. The results of Dhale's research [22] showed red pigment ranging from 59 OD.U/g moldy rice. Carvalho et al. [32] reported the result on the Angkak pigment extract from fermented rice having a pigment content of 216 OD.U/g dry substrate. This difference in pigment content is due to several types of substrate and substrate environmental conditions [33] and different methods of angkak pigment extraction.

3.2. Bioactive components

3.2.1. The component of purple sweet potato anthocyanin

The purple sweet potato anthocyanin component was analyzed using LC-MS. LC-MS separated the compounds in the anthocyanin based on their relative molecular mass indicated by the values of \([M]^+\) m/z ions and their fragmentation ions. Results of LC-MS chromatogram analysis (Chromatogram image not shown) main component of purple sweet potato anthocyanin is shown in Table 2.
Referring to the results of Montilla et al. [24], the anthocyanin component of purple sweet potato can be interpreted. Based on Table 2, extract of crude anthocyanin at \( t_R \) 3.7 minutes had a peak molecular ion of \([M]^+\) of \( m/z \) 773 with fragmentation ion of \([M-X]^+\) of \( m/z \) 611, 449, 287 which confirmed the compound anthocyanidin of cyaniind-3-sophoroside-5-glucoside. At the \( t_R \) of 17.5 minutes the chromatogram exhibited the cyanidin-3- (6’’ caffeoylsophoroside) -5-glucoside compound on peak ion of \((M)^+\) of \( m/z \) 935 with its fragmentation ions of \([M-X]^+\) were \( m/z \) 773, 449, 287. The anthocyanidin of cyanidin-3- (6’’ - caffeoyl-6’’- feruloylsophoroside) -5-glucoside were presented at peak of \([M]^+\) of \( m/z \) 1111 and at its fragmentation ion of \( m/z \) 949,449,287 with \( t_R \) of 18.3 minutes. While at peak of \([M]^+\) of \( m/z \) 1111 with its fragmentation ion of \([M-X]^+\) of \( m/z \) 949,463,301 at \( t_R \) 20 minutes, the chromatogram presented anthocyanidin in the form of peonidin-3- (6’’, 6’’- dicaffeoylsophoroside) 5-glucoside. At \( t_R \) of 21.5 minutes at peak ion of \([M]^+\) of \( m/z \) 1125 and at its fragmentation ion of \( m/z \) 963, 463, 301, the chromatogram confirmed the anthocyanin component of pn-3- (6’’ - caffeoyl-6’’ - feruloylsophoroside) -5-glucoside. Thus the major anthocyanin components present in purple sweet potato extract were cyanidin and peonidin. This is in accordance with the study of Montilla et al. [24]. Cyanidin (\( p_n / c_y < 1 \)) is an aglycone that produces blue color in anthocyanins in purple sweet potato cultivars, while red color is produced by peonidin (\( p_n / c_y > 1 \)) [34;35].

### Table 2. Characteristics of Mass Spectra of main component of purple sweet potato anthocyanin by LC-MS

| \( t_R \) (retention time, minutes) | \([M]^+\) (m/z) | Fragment (m/z) | Component |
|----------------------------------|----------------|----------------|-----------|
| 3.7                              | 773            | 611, 449, 287  | cy-3-soph-5-glc |
| 15.6                             | 1055           | 893, 449, 287  | cy-3-(6’’-caffeoyl-6’’-glucoside)-5-glc |
| 17.5                             | 935            | 773, 449, 287  | cy-3-(6’’-caffeoylsophoroside)-5-glc |
| 18.3                             | 1111           | 949, 449, 287  | cy-3-(6’’-caffeoyl-6’’-feruloylsophoroside)-5-glc |
| 20.0                             | 1111           | 949, 463, 301  | pn-3-(6’’, 6’’-dicaffeoylsophoroside)-5-glc |
| 21.5                             | 1125           | 963, 463, 301  | pn-3-(6’’, 6’’-dicaffeoyl-6’’-feruloylsophoroside)-5-glc |

**Note:** \( c_y \) = cyanidinid, \( s_o p_h = \) shoporoside, \( g_l c = \) glucoside, \( p_n = \) peonidin

### 3.2.2. The component of turmeric curcuminoid

Interpretation of major components in curcuminoid refers to the research of Jiang et al. [23] and Lee et al. [36]. Chromatographic analysis results (chromatogram not shown) component of curcuminoid extract with LC-MS in this study is shown Table 3.

### Table 3. Characteristic Mass Spectra of main component of curcuminoid turmeric by LC-MS

| \( t_R \) (minutes) | \([M+H]^+\) (m/z) | Fragment (m/z) | Component |
|--------------------|-----------------|----------------|-----------|
| 12.7               | 309             | 291, 239, 225, 215, 189, 147, 145 | Bisdemethoxycurcumin |
| 18.3               | 339             | 321, 269, 255, 245, 229, 177, 147 | Demethoxycurcumin |
| 19.5               | 369             | 351, 299, 285, 259, 245, 177, 175 | Curcumin |

Table 3 shows that at \( t_R \) 12.7 minutes legible the positive mode ion peak of \([M+H]^+\) of \( m/z \) 309 with its fragment ion was \( m/z \) 291, 239, 225, 189,144,144 confirming the curcumonoid component of bisdemethoxycurcumin. At \( t_R \) of 18.3 minutes, the chromatogram appeared the peak ion of \([M+H]^+\) of \( m/z \) 335 and its fragment ions were \( m/z \) 321, 269, 255, 245, 229, 177, 147 so the exhibited component was demethoxycurcumin. At \( t_R \) of 19.5 minutes, the peak of \([M+H]^+\) of \( m/z \) 351 and its fragment ions of \( m/z \) 351, 299, 285, 259, 245, 255, 177, 175 confirmed the curcumoid component of curcumin. Based on the results of the analysis using the LC-MS, it can be concluded that the main components of curcuminoid were curcumin, demethoxycurcumin, and bisdemethoxycurcumin.
3.2.3. The component of chlorophyll Suji leaf
The result of LC-MS chromatogram analysis of chlorophyll component (Figure chromatogram is not shown) is described in Table 4. Table 4 shows that at \( t_R \) 21.7 minutes, crude chlorophyll extracts had molecular ion peak of [M+H]+ of m/z 907 with its fragmentation ion of m/z 626, 597, 569, it confirmed chlorophyll component of chlorophyll b. At \( t_R \) 25.8 minutes, the chromatogram exhibited peak molecular ion of [M+H]+ of m/z 893 with fragmentation ion were m/z 615, 583, 555, it confirmed chlorophyll component in the form of chlorophyll a. Thus the chlorophyll of suji leaf extract is composed by two main pigment components namely chlorophyll a and chlorophyll b. This is in accordance with Paglia [37]. Chlorophyll a contributes to giving a bluish-green color in the leaf tissue whereas chlorophyll b gives the yellowish green color found with chlorophyll a in the plant [38]. In this study, the ion peak of [M+H]+ of m/z 893 had abundance only about 6% and its fragmentation ion of m/z 615, 583, 555 had abundance ranged 8%, 68%, and 5%, respectively. In the MS/MS of chlorophyll b, the peak ion of [M+H]+ of m/z 907 had abundance of 45% with its ion fragmentation of m/z 626, 597, 569 had abundance of 54%, 31%, and 4 %, respectively.

| \( t_R \) (min) | [M+H]+ m/z | Fragment (m/z) | Component |
|-----------------|------------|----------------|-----------|
| 21.5            | 907        | 626, 597, 569  | Chlorophyll b |
| 25.8            | 893        | 615, 583, 555  | Chlorophyll a |

3.2.4. Components of Angkak pigment
The result of LC-MS of Angkak pigment component (chromatogram image is not shown) is shown in Table 5. Table 5 shows that the chromatogram showed the peak ion of [M+H]+ of m/z 357 at \( t_R \) 17.5 minutes with abundance of 6%, it confirmed the pigment component of rubropunctatin. The \( t_R \) of 22.6 minutes had molecular ion of [M+H]+ of m/z 359 with an abundance of 51% confirming the components of pigment Angkak extract of monascin. The molecular ion peak of [M+H]+ of m/z 387 at \( t_R \) 23.4 minutes with percentage abundance of 7% showed the pigment component of ankaflavin. Other pigment component read from this LC-MS result was monascorubrin which was shown at peak of [M+H]+ of m/z 385 at \( t_R \) of 25.6 minutes with percentage abundance of 19%. Thus the components of Angkak pigment are composed of rubropunctatin, monascin, ankaflavin, and monascorubrin. This is in accordance with the results of Moussa and Azeiz [39] research. Rubropunctatin and monascorubrin are the main pigments of orange-shaped coloring in Angkak, while monascin and ankaflavin are yellow [40-41].

| \( t_R \) (minutes) | [M+H]+ m/z | Component |
|--------------------|------------|-----------|
| 17.5               | 357        | Rubropunctatin |
| 22.6               | 359        | Monascin |
| 23.4               | 387        | Ankaflavin |
| 25.6               | 385        | Monascorubrin |

3.3. The sensitivity of bioactives to pH 2-13
3.3.1. The sensitivity of anthocyanin to pH 2-13
Anthocyanin stability is influenced by pH, temperature and light intensity [42]. The result showed a significant, varying change of color of the anthocyanin in various pH condition. The color changed from red, purple, blue, green to yellow (from acid to alkaline solution). Anthocyanin is relatively stable at acidic condition than at neutral or alkaline condition. The result is shown in Figure 1(a).
Figure 1 (a) shows that anthocyanin was relatively stable with red color around acidic condition (pH 2-4) and then it changed into purple at pH 5-6, slowly turned into blue at pH 7, 8, 9, gradually changed into green at pH 10 and 11, and finally at pH 12-13 it turned into yellow. Malien-Aubert et al. [43] explained that the stability of purple sweet potato’s anthocyanin at acidic and neutral condition was due to the presence of predominant acylated cyanidin and peonidin in purple sweet potato. According to Lu et al. [44], the degradation of anthocyanin color at alkaline condition was as a result of the oxidation of phenolic hydroxyl anthocyanin in alkaline condition. The color of purple sweet potato’s anthocyanin extract changed as the pH swings. It was due to change of anthocyanin structure from flavylum cation into carbinol hemiketal pseudobase, quinonoidal base or chalcone [45-46]. At pH 1-2, anthocyanin was stable at reddish color as it was dominantly in the form of flavylum cation. At pH < 6 it changed into carbinol and partly changed into quinonoidal which was blue in color. At pH 6.5-9, the anthocyanin was blue as the dominant form of quinonoidal, while at pH >9 it was dominantly in the chalcone structure which was yellow in color[46].

Figure 1. The anthocyanin color changes from pH 2-13 (a), and the anthocyanin UV-VIS spectra at pH 2-13 in the range λ 400-800 nm (b)

The influence of pH to the anthocyanin color stability was investigated by the absorbance of UV light using UV-VIS spectrophotometer. The color change at pH 2-13 was indicated by the change of the distinctive absorbance at a certain maximum wavelength for each sample at each pH condition. Figure 1(b) displays the absorbance analysis using UV-VIS at wavelength range of 360-800 nm for sample with pH 2-13. Figure 1(b) shows that the spectrum of anthocyanin was shifted as the pH change. At pH 2-4, anthocyanin had a peak absorbance at wavelength of 520 nm. After the pH increased to 4-7, a bathochromic shift occurred from 520 nm (pH 4) to 540 nm (pH 5-7). This shifting exhibited the color change from red to purple and blue at pH 4-7. At the wavelength of 520, the color intensity increased at sample of pH 8-10, while decreased at the sample with pH 11-13. The bathochromic shifting occurred at pH 7-13 from the wavelength of 540 nm (pH 7) to 560 nm (pH 8-9), 580 nm (pH 10), and finally to 600 nm (pH 11-13). It exhibited the color change from blue into green and yellow (from pH 7 to 13). The spectrum shift took place due to the structure change of anthocyanin from flavylum cation to hemiketal or quinonoidal [47].
3.3.2. The sensitivity of curcumin to pH 2-13

In this research, we observed the color change of curcumin from pH 2 to 13. At acidic condition and neutral, the curcumin was relatively stable with no color change. Meanwhile, when it was in alkaline condition, the color changed from yellow into brownish-orange. As shown in Figure 2 (a), at pH 2-7 curcumin was stable in yellow color while at pH 8-13 it turned into brownish-orange. This concluded that curcumin would be stable when kept in acidic condition and the color would degrade at alkaline condition. This result is in accordance with the research of Tonnesen and Karlsen [48] and Pourreza and Golmohammadi [49]. According to Anand et al. [50], in acidic and neutral media, curcumin was dominantly keto, while in alkaline condition it was in the form of enol. The keto form determined the yellowish color, while the enol form was the cause of color degradation into brownish-orange.

Tonnesen and Karlsen [48] reported that curcumin was degraded by the effects of pH and produced derivative products of one of the feruloyl methane. According to Stankovic [51], feruloyl methane rapidly formed a yellow-colored condensation product yellow to brown. The spectrum of curcumin extract encountered a shifting as the pH changed. At pH 2-10, curcumin had a peak absorbance at the wavelength of 420 nm. For the sample with pH 13, bathochromic shift occurred from 420 nm (pH 10) to 440 nm (pH 11) and 460 nm (pH 12, 13). This alteration exhibited the color change from yellow to brownish-orange. The spectrum absorbance of curcumin at pH 2 to 13 at λ of 280-620 nm is shown Figure 2(b). This is in accordance with previous research of Pourreza and Golmohammadi [49].

![Figure 2](image_url)

**Figure 2.** The curcumin color changes from pH 2-13 (a), and the curcumin UV-VIS spectra at pH 2-13 in the range λ 280-620 nm (b)

3.3.3. The sensitivity of chlorophyll to pH 2-13

Figure 3a shows that chlorophyll had color degradation when reacting with acidic pH, while the color of chlorophyll remained green when it reacted with an alkaline pH. At pH 2-5 the color of chlorophyll turned from green to green olive (yellowish green), and at pH 6-13 the color of chlorophyll was stable in green. Some researchers reported that the chlorophyll green color loss was a result of the influence of pH and heat marked by color change from green to green-olive [52-53]. According to Gold dan Weckel [52], degradation of chlorophyll color by acidic pH occurs due to conversion of chlorophyll to feofitin. Minguez-Mosquera et al. [54] mentioned that acidic pH can remove magnesium ions (Mg2+) presenting in the center of the porphyrin ring and it is replaced with hydrogen ion to form feofitin. According to Greve et al. [55] and Van et al. [56], the conversion of chlorophyll to feofitin and pheophorbide causes a change of color from bright green to olive yellow, resulting in a decrease in the quality of chlorophyll.
Figure 3. The chlorophyll color changes from pH 2-13 (a), and the chlorophyll UV-VIS spectra at pH 2-13 in the range λ 280-620 nm (b).

Chlorophyll spectra at pH 2-13 is shown in Figure 3 (b). Figure 3 (b) shows that the absorption of chlorophyll color at pH 2-13 had two wave peak for the absorption of green light that was at λ 400-420 nm and 640-680 nm. At pH 2, the reflection of green light occurred at λ 420 nm and 680 nm. At pH 2-3 occurred hypochromic shift of 20 nm for blue light absorption, it was λ 420 nm (pH 2) to λ 400 nm (pH 3). At pH 4, green light reflections occurred at λ 420 nm and 680 nm. The bathochromic shift occurred from pH 3 to 4 being to a position such as pH 2. At pH 5-11, the maximum absorbance occurred at λ 420 nm and 660 nm, so that the hypochromic shift of red light wavelength occurred at pH 4-11 from λ 680 nm (pH 4) to λ 660 nm (pH 5-11). Hypsochromic shift also occurred at pH 11-13 from λ 660 nm (pH 11) to λ 640 nm (pH 12-13). The wavelength shift occurring from pH 2-13 showed a change in chlorophyll structure due to the influence of pH.

3.3.4. The sensitivity of Angkak pigment to pH 2-13

Figure 4 (a) shows that at pH 2 there was a change of pigment color from red-orange pigment to yellow. At pH 3-9, the color of Angkak pigment remained stable in red-orange, and at pH 10-11 the color slightly faded into pale red-orange, while at pH 12-13 Angkak pigment turned yellow. This showed that Angkak pigment was unstable at acidic pH and alkaline pH of pH > 9 and it was stable at neutral until alkaline of pH ≤ 9. It showed that Angkak pigment was stable at pH 3-11 while below of pH 3 and above of pH 11 Angkak pigment was rapidly degraded from red-orange to yellow. This is in accordance with Kaur et al. [57] where degradation of Angkak pigment occurred rapidly below of pH 4 and above of pH 8. According to Fabre et al. [58], the good stability of Angkak pigment is at pH 6 to 8.

The red dry orange pigment color change from red-orange to yellow at pH 2 and 12,13 is probably caused by the breakdown of the chromophore group structure on the main pigment of Angkak (monascorubrin and robrupunctatin) causing the red-orange pigment to turn into a derived pigment of monascin and ankaflavin form a yellow color.
Figure 4. The Angkak pigment color changes from pH 2-13 (a), and pigment Angkak UV-VIS spectra at pH 2-13 in the range λ 280-620 nm (b)

Angkak pigment spectra were shown in Figure 4 (b). Figure 4 (b) showed that there was a bathocromic shift of pH 2-11 at λ 400 nm (pH 2) to λ 500 nm (pH 3-11) and at pH 11-12 hypsoocromic shift occurred from λ 500 nm (pH 11) to λ 400 nm (pH 12) then bathocromic shift occurred from λ 400 nm (pH 12) to λ 480 nm (pH 13). Then there was a shift again at pH 11-13 because there was a change of Angkak pigment from red-orange to yellow. The shifting of wavelength indicates a change of Angkak pigment structure due to the presence of pH which causes the change of color.

4. Conclusions
Anthocyanin and curcumin were the best bioactives as pH biosensor because they provided a unique and significant color changes compared to chlorophyll and angkak pigment that did not show clear discoloration. The anthocyanin was sensitive to pH 5-13 and curcumin was sensitive to pH 7-13 and both were stable at acidic pH. The selectivity and sensitivity of bioactive to pH are the basic information in the development of biosensor. Therefore, anthocyanin and curcumin are potential to be developed as biosensor for pH detection.

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