The study of spectral uv-vis on coconut water as flour solvent of soybean, black rice and red rice

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Abstract. Coconut water (CW) is a nutritious drink containing highly complex components. Therefore, CW has an interesting unique physicochemical nature to investigate. This study aims to know the UV-Vis spectra of CW as flour solvent of soybean, black rice, and red rice. Each flour was homogenized with ratio of 1:10 (w/v) in fresh CW (f-CW) or heated-CW at 50 ºC (h-CW). The homogenized flour was centrifuged at 4500 rpm, 4ºC, for 5 minutes. The supernatant was analyzed based on UV-vis spectra, pH, and turbidity. Flour that dissolved in CW shows peaks only in the UV spectrum, whereas the flour dissolved in water shows peaks in the UV-vis spectrum. Generally, flour component more dissolves in the CW than in the water. Turbidity value of flour dissolved in the CW lower than those dissolved in the water. The flour dissolved in h-CW shows a slight decrease in absorbance value compared to the f-CW, meanwhile, flour dissolved in water generally have relatively the same spectrum profile.

Keyword: black rice, coconut water, red rice, soybean, UV-Vis spectrums

1. Introduction

Coconut water (Cocos nucifera) is a rich nutrient isotonic water [1] with clear appearance, good taste, low calories, and therapeutic potential. This is due to the ability of coconut water to restore electrolytes in cases of dehydration [2]. Coconut water can easily change when exposed to air. These changes are characterized by a special reaction carried out by polyphenol oxidase and peroxidase which has an impact on changes in nutrient content, taste, pH, and odor of coconut water [1,3]. Nevertheless, coconut water has a high enough selling potential because many people use it as a refreshing traditional drink [4]. Besides, coconut water is also used as a mixture in a polyherbal EMSA Eritin [5].

EMSA Eritin is an Indonesian polyherbal in the form of a food supplement made of a mixture of red rice, soybeans and coconut water [5]. Red rice has a polyphenol content of up to 81% which can act as an antioxidant [6]. However, black rice (Oryza sativa L. indica) is a rice type that reported having higher anthocyanin content than those red rice [7]. Soy protein isolate, when conjugated with black rice anthocyanin extract, has higher digestibility than soy protein isolates only. The antioxidant ability of soy protein isolates added with anthocyanins has increased, so that it can be a healthy
alternative [8]. This allows the use of black rice as a polyherbal formulation of EMSA Eritin replacing the red rice.

Polyherbal testing is challenging because all herbs or herbal products are considered to be an active ingredient, regardless of whether they have a therapeutic activity or not [9]. studies related to physicochemical characters have not been widely used, especially in coconut water, soybeans, black rice, and red rice. One way to find out the physicochemical character is by looking at the interaction of the active material in it. The interaction of active material in an herbal formula can be detected using UV-Vis spectrophotometry, which is by looking at changes in spectrum profiles that indicate interactions [10]. The interactions formed can be used to determine the complex stability and standardization of polyherbal quality. Therefore, this study was conducted to describe the molecular interactions that occur in coconut water and soybean complexes with red rice and black rice through the UV Vis spectrum produced. The purpose of this study is to describe the UV Vis spectrum of coconut water as a solvent for soy flour, black rice, and red rice.

2. Method

2.1. Flour Making
This research used organic red rice Inpari 24, black rice Woja Laka, and soybean Devon from Balai Penelitian Kacang dan Umbi-umbian in Malang. Dried-rice weighed 200 g each, milled using a blender, sifted using a 0.25 mm (60 mesh) sieve.

2.2. Coconut water
The coconut used was 7 months old green coconut fruit. The mesocarp of the coconut fruit was perforated using a knife. Water from coconut fruit was poured into a glass beaker. Coconut water was filtered using filter paper before used (Manjunatha and Raju, 2013). Coconut water separated into two different containers for fresh coconut water and fresh coconut water heated at 50 °C.

The next step is making polyherbal using coconut water or distilled water (as a comparison). The solution for first homogenization uses a ratio of 1:10 (w / v), 10 g of dissolved material was dissolved in 100 ml of solvent. Homogenization repeated 3 times. The mixture was stirred until the flour dissolved, then homogenized with a blender for 1 minute. The flour dissolved in the solvent without heat treatment, soaked for 2 hours in the solvent before being homogenized. Each solution was centrifuged at 4500 rpm for 15 minutes at 4 oC. The centrifugation method refers to Alghamdi et al. (2018) [11]. The supernatant from the centrifugation was taken as a sample, while the pellet was resuspended with 75 ml of the solvent and then centrifuged again. The pellets were resuspended with 50 ml of solvent to continue third homogenization. Supernatants from all centrifugation were accumulated and analyzed for UV-Vis spectrum profile.

2.3. UV-Vis Spectrum Analysis
Samples were taken as much as 3 mL and put into cuvettes. The samples were analyzed for absorbance patterns using the Genesys 10uv UV-Vis spectrophotometer in the wavelength range of 190-790 nm. Each sample was tested 3 times. The results of the analysis taken to made graphs using Microsoft Excel, the wavelength is plotted on the X-axis, while the absorbance value is plotted on the Y-axis.

2.4. Measurement of pH and Turbidity
Measurement of turbidity was carried out using a turbidimeter (Hach 2100q). The sample was inserted into the sample bottle to the boundary line on the bottle. Then turbidity measurements are carried out. PH measurements were then carried out using a pH-meter (Qis4). the tip of the pH meter electrode was inserted into the sample. Steps were repeated for different samples. Furthermore, all data obtained were presented descriptively.
3. Result and Discussion

3.1. UV-Vis spectrum Solution of soybean flour, black rice, and red rice

The UV spectrum of samples is at wavelengths of 190-390 nm and Vis spectra at wavelengths of 390-790 nm. However, in soybean flour samples dissolved by distilled water (S1), mixed soybean and red rice (SRR1) or black rice (SBR1) flour dissolved by distilled water showed absorbance values without any peak, both in the UV and Vis regions. The sample of red rice flour dissolved by distilled water (RR1) has a UV-Vis spectrum profile with 6 peaks while black rice flour dissolved distilled water (BR1) has 5 peaks (Figure 1). The appearance of this peak shows the presence of chromophore groups from red rice and black rice flour. The profiles formed on BR1 generally tend to be the same as the RR1 spectrum profile with several peak shifts. The appearance of peaks in the RR1 and BR1 samples is tabulated in table 1. The spectrum profile shown by SBR1 and SRR1 samples resembles the spectrum profile of S1 samples. This similarity shows the dominance of soybeans against red rice and black rice because the UV-Vis spectrum profile of red and black rice flour dissolved in distilled water has a different UV-Vis spectrum profile.

![Figure 1. UV-Vis spectrum of flour dissolved in distilled water](image)

Based on the results of the study it was alleged that distilled water can dissolve some of the components found in red rice and black rice flour. The dissolved component is in the form of molecules that can absorb light energy known as a chromophore. The chromophore is a molecule that has a functional group and able to absorb UV and Vis light at certain wavelengths. The absorption of energy causes the transition of electrons in the outer shell to a higher energy level [12] so that the peak of absorbance emerges. While the absence of peaks in the samples S1, SBR1 and SRR1 were caused by the concentration of the solution was too high, so that the profile cannot be read on the device. Soybeans contain more protein than rice and protein in soybeans more soluble than rice [13]. The ability of soybean components to dissolves higher compared to rice possibly influenced the absorbance and character of the spectrum formed in SBR1 and SRR1 which is dominated by the soybean spectrum.

3.2. Effect of Coconut Water on Sample UV-Vis Spectrum

The use of coconut water caused changes in the UV-Vis spectrum in all samples. Black and red rice flour dissolved with distilled water (BR1 and RR1) produce peaks in the UV and Vis area while black rice and red rice flour dissolved in coconut water (BRC1 and RRC1) only produce a peak in the UV
area (Figure 2). The BRC1 and RRC1 peak formed in the UV area tend to be higher than those in samples BR1 and RR1. The BRC1 and RRC1 have lower absorbance than samples BR1 and RR1. Overall, samples using coconut water solvents showed lower absorbance values compared to samples using distilled water solvents.

Table 1. Location of peak samples in the UV and Vis regions

| Sample | UV | Vis |
|--------|----|-----|
|        | 220-230 | 221-231 | 225-250 | 227-270 | 271-280 | 281-300 | 300-330 | 331-360 | 361-390 | 391-490 | 491-590 | 591-690 |
| S1     | -    | -    | -    | 289     | -    | -    | -    | 389     | -    | -    | -    | -    |
| BR1    | -    | -    | -    | -    | 296   | -    | -    | 362     | 420   | -    | 486   | 529   |
| RR1    | -    | -    | -    | -    | 289   | 311   | 359   | -    | 420   | 483   | 529   |
| SBR1   | -    | -    | -    | 289   | -    | -    | 389   | 486     | 589   | -    | 677   |
| SRR1   | -    | -    | -    | -    | 289   | -    | 389   | -    | -    | -    | -    |
| C1*    | 229  | 262  | 271  | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| BRC1   | 229  | 247  | 262  | 274  | 286   | 296   | -    | -    | -    | -    | -    | -    |
| RRC1   | 226  | 250  | 262  | 286  | 289   | -    | -    | -    | -    | -    | -    | -    |
| SC1    | -    | -    | -    | -    | 289   | -    | -    | -    | 389   | -    | -    | -    |
| SBRC1  | 232  | 247  | 262  | 274  | 289   | 296   | -    | -    | -    | -    | -    | -    |
| SRR1   | 232  | 250  | 262  | 286  | 289   | -    | -    | -    | -    | -    | -    | -    |

*referring to the results of the Ekasari study (2019).

The BRC1 samples peaks from coconut water peak at wavelengths 229, 262 and 274 nm, and peaks of black rice at 296 nm. This combination produces a new peak at wavelength 247 and 286 nm. The SBRC1 samples also tend to be alike BRC1 samples but with peak, shifts originating from coconut water from 229 nm to 232 nm and from 271 nm to 274 nm. Peak 289 nm in SBRC1 derived from soybeans and new peaks in this combination are also the same as BRC1 samples formed at 247 nm. The use of coconut water as a solvent in a mixture with soybeans and black rice shows an interaction between the components of soy and black rice flour with coconut water. Coconut water can increase the clarity of the mixture with soy and black or red rice. It can be seen from the lower spectrum in the visible light region and the solution is clearer than those in the distilled water solvent. Some peaks in SBRC1 undergoing bathochromic shift when compared to BRC1 samples. Changes in the form of additions and shifts in the peak indicate the interaction of coconut water and black rice flour with the addition of soy flour.

Based on the peak location data of each sample, it is known that the peak on SRR1 poly-herbal consists of characteristics of new peak and mono-herbal peaks. Peak which is characteristic of mono-herbal and appears in SRR1 poly-herbal, among others: peak at 262 nm wavelength that appears at coconut water, peak at 289 nm wavelength that appears on S1 and RR1. The new peak that appears on polyherbal is the result of peak shift and solubility of other components. Peak RRC1 at 226 nm undergoes a shift towards a higher wavelength on SRR1 poly-herbal, which is a wavelength of 232 nm.
Figure 2. UV-Vis spectrum of flour with aquadest and coconut water solvent, (a) single flour sample (b) combined flour sample.

The peak shift that occurs in the sample is caused by the conjugation of two individual chromophores. When more than one chromophore (both the same and different types of chromophore) conjugates, multiple chromophores will form. The formation of multiple chromophores can cause changes in the absorbance intensity to be higher (hyperchromic), shifting the absorbance wavelength towards the higher wavelength, and the formation of absorbance at the new wavelength. The movement of peak absorbance towards a higher wavelength is called bathochromic shift. The greater the conjugation rate or the number of binding chromophores, the higher the bathochromic and hyperchromic effects [14].

3.3. Effect of Solvent Heating Treatment on UV-Vis Spectrum Profile

The treatment of heating coconut water to a temperature of 50°C has a various effect on polyherbal solutions. Heating causes peak shifts, decreases, and increases in absorbance values. In RRC2, the use of heated coconut water causes peak shifts towards higher and lower wavelengths, while in BRC2
there is a peak shifting towards lower wavelengths, from 274 to 271 nm (Table 2). In the SC2, SBRC2, and SRRC2, the profile generally did not change, but the heating treatment caused a slight decrease in the absorbance value. The profile of the BRC2 spectrum also tends to be the same, but there is a slight increase in the absorbance value (figure 3).

The solvent heating process was also carried out on distilled water solvents. Heating distilled water also shows various effects on polyherbal. the use of distilled water with a temperature of 50°C causes the formation of 3 new peaks in RR1. The formation of new peaks in samples of RR2 is thought to be due to the presence of other components of red rice flour which can be dissolved when added with a solvent heated to a temperature of 50°C. Black rice flour dissolved using distilled water 50°C (B2) shows an increase in absorbance in the visible light region and a shift in peak absorbance towards shorter waves. While S2, SBR2, and SRR2 samples did not change the UV-Vis spectrum profile, the sample encountered a slight decrease in absorbance value (figure 4). Peak shifts in each sample can be tabulated in table 2.

Figure 3. Effect of solvent heating treatment on UV-Vis spectrum profile of flours dissolved in coconut water.
Figure 4. Effect of solvent heating on the UV-Vis spectrum profile of flour dissolved with distilled water.

Table 2. Shifts and formation of new peaks in flour solutions

| No | Samples                                      | Peak shift                  | λ of new peak (nm) |
|----|----------------------------------------------|-----------------------------|--------------------|
|    |                                              | To a shorter wavelength     | To longer          | 247 | 250 | 253 | 274 |
| 1  | Red rice flour dissolved in distilled water  | 311 → 289                  | -                  | ✓   | ✓   | ✓   |
| 2  | Red rice flour dissolved in coconut water    | 250 → 247                  | 226 → 229          | -   | -   | -   | -   |
| 3  | Black rice flour dissolved in distilled water| -                           | 296 → 299          | ✓   | -   | -   | -   |
| 4  | Black rice flour dissolved in distilled water| 274 → 271                  | -                  | -   | -   | -   | -   |
3.4. Solution turbidity

The difference in absorbance values in all samples in the Vis area is thought to be related to the value of turbidity. Visually, all the turbidity levels can not be distinguished by turbidimeter. The turbidity value of the solution is directly proportional to the absorbance value produced in the Vis spectrum. If the turbidity value $> 1000$ NTU, the absorbance value is $> 0.5$ and if the turbidity value is $< 1000$ NTU, the absorbance value is $\leq 0.5$ at the wavelength of 660 nm [15]. The entire sample used tends to have a turbidity value of $> 1000$ NTU except for samples BAmk1, BAmk2, and KBAk2 which have a turbidity value of $< 1000$ NTU.

The results of measurements of samples turbidity show samples dissolved using distilled water, i.e. BR1, BR2, RR1, RR2, SBR1, and SBR2, have turbidity above 1000 NTU (Table 3). Based on the effect of the type of solvent, the turbidity between BR1 samples is higher than SBR1, while for SBR1 and SBRC1 samples above 1000 NTU. The addition of soybean flour in the sample showed an increase in turbidity in the sample seen in SBR1 compared to SBRC1. The sample dissolved in distilled water is suspended so that it becomes cloudy and difficult to detect by turbidimeters. Heating in samples of black rice flour and soybeans with black rice dissolved in coconut water decreases absorbance and is confirmed at lower turbidity measurement results.

Variations in the value of turbidity are possible for different components of different coconuts. Based on the research conducted by Ekasari, (2019) it is known that coconut water from different coconuts has different turbidity values, ranging from 11 NTU to 31 NTU [16]. Fresh coconut water has a turbidity value of 17 to 31 NTU. The turbidity value can be tabulated in the following table:

| Sample code | Turbidity (NTU) | Sample code |
|-------------|----------------|-------------|
|             | $<1000$ | $>1000$ | $<1000$ | $>1000$ |
| S1          | -      | $\sqrt{\quad}$ | SC1      | -      | $\sqrt{\quad}$ |
| S2          | -      | $\sqrt{\quad}$ | SC2      | -      | $\sqrt{\quad}$ |
| BR1         | -      | $\sqrt{\quad}$ | BRC1     | $\sqrt{\quad}$ | -     |
| BR2         | -      | $\sqrt{\quad}$ | BRC2     | $\sqrt{\quad}$ | -     |
| RR1         | -      | $\sqrt{\quad}$ | SBRC-1   | -      | $\sqrt{\quad}$ |
| RR2         | -      | $\sqrt{\quad}$ | SBRC-2   | -      | $\sqrt{\quad}$ |
| SBR1        | -      | $\sqrt{\quad}$ | SBRC1    | -      | $\sqrt{\quad}$ |
| SBR2        | -      | $\sqrt{\quad}$ | SBRC2    | $\sqrt{\quad}$ | -     |
| SRR1        | -      | $\sqrt{\quad}$ | SRRC1    | -      | $\sqrt{\quad}$ |
| SRR2        | -      | $\sqrt{\quad}$ | SRRC2    | -      | $\sqrt{\quad}$ |

3.5. The pH of the solution

Flour dissolved in coconut water has a pH that tends to be more acidic than flour which is dissolved in distilled water (Table 4). This is because the pH of coconut water is classified as acidic pH. Changes in the pH value of the solution are thought to be due to the influence of the use of coconut water. The higher the pH value, the less activity of microorganisms in coconut water, and the lower the turbidity value. The low turbidity value causes a low value of absorbance produced.

Changes in solvents from distilled water to coconut water increase the pH of samples involving black rice flour while samples using soy flour and brown rice tend to decrease pH. Changes in the pH of insignificant solutions in all samples indicate the condition of the sample maintained, especially in coconut water. Coconut water is often used as a buffer for storing animal semen because it contains inorganic components that can maintain the survival of spermatozoa [17]. During World War II, coconut water was used for short-term intravenous hydration and resuscitation fluids [18]. This shows the ability of coconut water to maintain pH so that samples dissolved in coconut water become more stable than those dissolved by distilled water.

pH change is one indication of changes in the physicochemical properties of coconut water. Changes in the pH of coconut water to be more acidic indicate the occurrence of coconut water
fermentation due to the growth of microorganisms [19]. Based on the research conducted by Ekasari (2019), it is known that fresh coconut water has a pH of 5.55. The difference in pH values in each sample can be tabulated in the table below [16]:

| Sample code | pH    | Sample code | pH    |
|-------------|-------|-------------|-------|
| S1          | 6.87±0.06 | SC1 | 5.93±0.03 |
| S2          | 6.86±0.01 | SC2 | 5.93±0.23 |
| BR1         | 5.53±0.83 | SRC1 | 5.62±0.11 |
| BR2         | 5.70±0.74 | BRC2 | 5.70±0.16 |
| RR1         | 6.67±0.19 | RRC1 | 5.57±0.17 |
| RR2         | 6.94±0.03 | RRC2 | 5.57±1.19 |
| SBR1        | 5.52±0.86 | SBRC1 | 5.56±0.35 |
| SBR2        | 6.32±0.37 | SBRC2 | 5.86±0.12 |
| SRR1        | 6.85±0.11 | SRRC1 | 5.85±0.16 |
| SRR2        | 6.67±0.15 | SRRC2 | 5.54±0.25 |

4. Conclusion

Based on the results obtained, there is an interaction between coconut water and soy flour. black and red rice which influence the change in UV Vis spectrum profile that is formed. Black rice and red rice flour have more peaks in the UV area than soy flour which is dissolved in coconut water. The process of heating coconut water to a temperature of 50 degrees Celsius causes a peak shift and a decrease in the absorbance value in the sample used. The difference in the absorbance value in the sample correlates with the turbidity value. The use of coconut water as a solvent can cause a decrease in the pH of the sample.

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