The Physicochemical Properties of Annatto Extract Obtained from Distillate Water as Solvent Extraction in the Variation of pH and Extraction Time

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Abstract. Annatto is one of the most common natural colorants used in the food industry. The commercial processes to extract annatto were direct extraction into oil, direct extraction into aqueous alkali, or indirect extraction with solvents. The physicochemical properties of the extract are known to be influenced by the pH of the solvent and the extraction time. This study was conducted to investigate the physicochemical properties of annatto extract using distilled water as a solvent extraction. A randomized block design was employed with two factors, the pH of solvent (4, 7 and 9) and the extraction time (5, 7.5 and 10 min) with three replications. Extraction was carried out using the maceration method. The characteristics evaluated were soluble protein, reduction sugar, total soluble solids, and turbidity of extracts. The extraction with pH 7 of distilled water resulted in the highest soluble protein (5.40 mg/ml), reducing sugar (5.40 mg/ml), total dissolved solids (1.18 obrik), and absorbance of turbidity (λ 350) was 0.56. The annatto extraction for 10 min resulted in the highest dissolved protein (5.37 mg/ml); total dissolved solids (1.36 obrik) and turbidity absorbance (λ 350 nm) of 0.61, while the highest reducing sugar was produced for 5 min of extraction.

Keywords: Distilled water · Extraction · pH · Extraction time · Physicochemical properties

1 Introduction

Annato is a pigment produced from the seeds of Bixa orellana L. Annatto contains carotenoids (bixin and norbixin) which can be used as natural dyes that are safe and get recommendations from the FDA (Cabrera et al., 2015). The results of the research by Venugopalan and Giridhar (2012) and Yolmeh et al. (2012) showed that annatto extract has antimicrobial activity besides being a potential dye source. Abayomi et al. (2016) stated that annatto contains saponins, tannins, steroids and glycosides. The color characteristics of annatto extract in the form of brightness or color brilliance and color strength or purity are influenced by the components in the extract.

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Obtaining extracts from plants can be done by extraction. Extraction is a method for removing specific components from solids or liquids with a solvent (solvent). Extraction of solids (leaching) is a process of separating or taking the desired solid fraction from other solid fractions in a solid-solid mixture using a liquid solvent (Paryanto et al., 2014). Several methods of annatto extraction have been carried out, including using Sokhlet and maceration (Do et al., 2014). Annato extraction in stages by washing has also been carried out by previous researchers using hexane and followed by a polar solvent, namely acetone (Balaswamy et al., 2012). Other solvents used for annatto extraction include essential oil, hexane, chloroform, ethyl acetate, butanol, methanol and vegetable oil (Husa et al., 2018; Silva et al., 2008).

Factors that affect the extraction process include the extraction process temperature (Yuniawati, 2012), stirring speed (Anggista et al., 2019), as well as solvent pH, and extraction time (Paryanto et al., 2014). The pH of the solvent in the extraction process can affect the pH of the extract, the colour of the extract, the antibacterial activity, and the solubility of carotenoids and other components. Do et al. 2014; Hosain and Shah, 2015) stated that the colour and antioxidant characteristics obtained at the extraction stage depend not only on the extraction method used but also on the type of solvent used. Do et al. (2014) also stated that the annatto ethanol extract contained higher total phenols than the methanol extract. Extraction of annatto using distilled water is one of the profitable extraction methods because distilled water is a safe solvent, cheap, and easy to obtain.

In addition, the use of distilled water is known to produce a high intensity of annatto colour (Handayani et al., 2022). So far, physicochemical studies, especially soluble proteins, reducing sugars, total dissolved solids and turbidity of the annatto extract obtained by extraction using distilled water at variations in temperature and extraction time have not been carried out.

2 Materials and Methods

2.1 Annatto Extraction

The annatto extraction was carried out by Rosamah et al. (2013); Aboyami et al. (2014) and Handayani et al. (2022). A total of 25 g of cassava seeds were put into 90 mL of solvent. Maceration was performed using a magnetic stirrer with heating at 80 C and extraction time variations of 5, 7.5, and 10 min. In making the extract, distilled water was used as a solvent with variations in pH, namely 4, 7, and 9. The adjustment of pH four was carried out by adding citric acid, while pH nine was adjusted using Ca(OH)2.

After extraction, filtering was carried out using filter paper to separate the extract from the annatto seeds.

2.2 Turbidity

The turbidity of annatto extract was expressed as an absorbance value measured using a spectrophotometer at a wavelength of 350 nm. The annatto extract was diluted 100 x using distilled water as the diluent. The extract that has been diluted is then measured in the absorbance value.

2.3 Total Dissolved Solids (Muchtadi & Sugiyono, 1990)

Measurement of total dissolved solids using a hand refractometer at a temperature of 20 ºC and calibration using distilled water. A total of 1–2 drops of the sample are placed on a refractometer prism, and the amount of dissolved solids content is expressed as oBrix.

2.4 Soluble Protein (Sudarmadji et al. 1997)

Soluble protein was determined by the Lowry method using bovine serum albumin (BSA) as the standard. A total of 1 ml of the sample plus 8 Lowry B reagents were allowed to stand for 10 min. Furthermore, 1 ml of Lowry A was added to the solution and allowed to stand for 20 min. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 600 nm. The dissolved protein content of the sample was calculated using the standard curve of the BSA solution.

2.5 Reducing Sugar (Sudarmadji et al. 1997)

Reducing sugar analysis was carried out using the Nelson Somogyi method. The analysis begins with making a standard curve using glucose as the standard reducing sugar. Sample preparation was carried out by taking 1 ml of annatto extract and diluting it. A total of 1 ml of the diluted extract was added with 1 ml of Nelson's reagent, heated for 20 min, and then cooled. After cooling, 1 ml of arsenomolybdat reagent was added. 7 ml of distilled water was added to the sample. The optical density sample was measured using a UV-V is 1800 Shimadzu spectrophotometer at 540 nm. The reduced sugar content of the annatto extract was calculated based on the absorbance of the extract plotted on a standard curve.

3 Results and Discussion

3.1 Turbidity

Extraction of annatto seeds using distilled water is solid in liquid extraction. In the solid-in-liquid extraction process, there is an interaction between the solid and the liquid so that one or more molecules in the solid will move from the solid to the solvent to form a

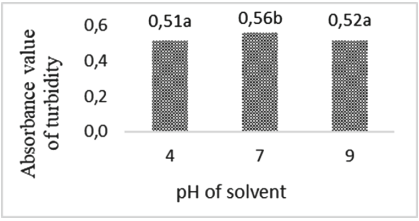

Fig. 1. The absorbance value of turbidity of Annatto extract in the variation of pH of the solvent.

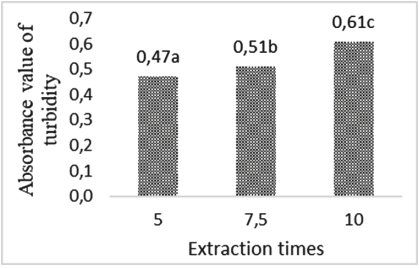

Fig. 2. The absorbance value of turbidity of annatto extract in the variation of extraction times.

solution or extract (solute and solvent). Factors that affect the extraction process include extraction temperature, solvent pH and extraction time ((Yuniwati, 2012); Paryanto et al., 2014). The effect of distilled water pH on turbidity is shown in Fig. 1.

Annatto extraction using distilled water pH 7 produces the highest turbidity (Fig. 1). Different turbidity indicates different concentrations of extracted compounds. At neutral pH, it dissociates into protons and hydroxyl ions under balanced conditions. The addition of citric acid and Ca(OH)2 used for pH adjustment causes a change in the polarity of the water, causing a change in the solubility of the compounds in the water. Adding acid or base causes a change in the dipole moment and dielectric constant of the water molecule so that the polarity of the water changes and causes the compound's solubility to change. Annatto contains reducing sugars, soluble proteins, the carotenoid norbixin, and polar flavonoids. Annato also contains non-polar tannins, phenols, and bixins. Annatto is thought to contain polar compounds in higher concentrations than polar compounds, so annatto extraction using water at pH 7 produces the highest turbidity. Three measures indicate the polarity of a solvent, namely the dipole moment, the dielectric constant, and its solubility in water. Molecules of solvents with large moment dipoles and high dielectric constants are polar (Rismawati and Ismiyati, 2017).

According to Mello et al. (2006), water is the most polar solvent with a dielectric constant of 80; with variations in pH in the water solvent used, the polarity of water decreases so that it can extract not only polar compounds in propolis but also semi-polar compounds. The effect of extraction time on the turbidity of annatto extract is shown in Fig. 2.

Figure 2 shows that increasing the extraction time increases the turbidity caused by more components in the material being extracted into the solvent. The increase in

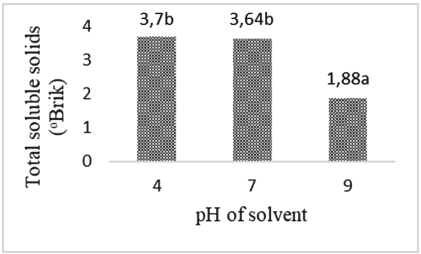

Fig. 3. Total soluble solids of annatto extract in the solvents of pH variation.

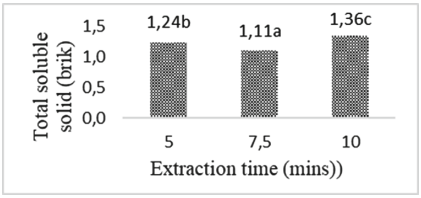

Fig. 4. Total soluble solids of annatto extract in the extraction times variation.

turbidity was caused by the increasing number of suspended and dissolved molecules in the extract. Annatto contains various compounds, including carotenoids, carbohydrates, proteins, flavonoids, saponins, tannins and phenols. These compounds cause turbidity in the annatto extract.

3.2 Total Soluble Solids

The annatto extraction was carried out using polar distilled water, so the extract's dissolved compounds were polar compounds. The total dissolved solids of annatto extract at various pH are shown in Fig. 3.

Annatto extraction in acidic solvents is thought to cause hydrolysis of carbohydrate compounds in annatto. Gupta (2016) stated that annatto seeds contain sucrose. Sucrose is a non-reducing sugar that can be hydrolyzed into reducing sugars by acids. Astuti et al. (2018) stated that the increase in total dissolved solids could be due to the hydrolysis of carbohydrates into soluble sugars. The increase in total dissolved solids can also be caused by an increase in the temperature and extraction time. The total dissolved solids content of annatto extract at various extraction times is shown in Fig. 4.

The highest total dissolved solids were produced at 10 min of extraction and the lowest at 7.5 min. The formation of complex compounds resulting from the reaction of proteins and reducing sugars is thought to occur during extraction. During extraction, it is suspected that there will be changes in reducing sugars. Ten mins of extractions are thought to cause hydrolysis of complex carbohydrates into sugars. While in 7.5 min of extraction, it is suspected that there was a reaction between the reducing sugar and the metals or minerals present in the annatto extract, which caused a decrease in the reducing sugar. Gupta (2016) stated that annatto seeds contain sucrose. Sucrose is a non-reducing sugar that can undergo hydrolysis into reducing sugars.

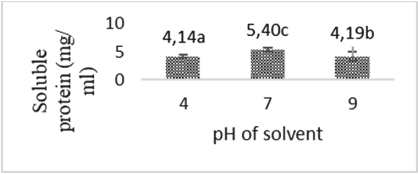

Fig. 5. The total soluble protein of annatto extract in the solvents of pH variation.

Factors that affect hydrolysis include pH, temperature and reaction time. The annatto extraction carried out at a temperature of 80 °C is suspected of causing hydrolysis of carbohydrates and the reaction of complex formation between protein and carbohydrates. Extraction for 7.5 min is thought to cause a molecules complex between sugar and protein, which causes low total dissolved solids. On the other hand, extraction at 10 min caused the hydrolysis of sucrose in annatto which caused an increase in reduced sugar content.

3.3 Soluble Protein

Proteins are amphoteric molecules that can be positively or negatively charged, depending on the pH of the solution. The charge on the protein contributes to the increase in protein solubility (Kristianto et al., 2019). The effect of solvent pH on the dissolved protein content of annatto extract is shown in Fig. 5.

The highest protein solubility was produced in distilled water at pH seven and the lowest at pH 4. From the analysis of protein solubility, it was suspected that annatto extract contained polar amino acids with positive and negative charges and uncharged side groups. It is suspected that the content of uncharged amino acids is found in the highest amount, followed by positively charged amino acids and the least negatively charged amino acids. The high uncharged amino acids in annatto cause no interaction with protons and hydroxyl ions in water molecules, so the solubility remains high. The polar amino acids include serine, cysteine, threonine, methionine, asparagine, and glutamine. The presence of negatively charged amino acids in annatto causes an interaction between the negative group on the amino acid (COO^-) reacting with the proton (H^+) in water added with citric acid, resulting in protein deposition, which causes low solubility. Negatively charged amino acids are aspartic acid and glutamate (Lehninger). Adding acid or base causes a change in the charge balance in water so that protons from citric acid or hydroxyl ions from calcium hydroxide or water bind to amino groups or carboxyl groups on amino acids, which causes the globular protein to unfold. The unfolding of protein globular structure causes a decrease in protein solubility (Pace et al., 2004).

The protein in annatto which is polar and uncharged causes a higher protein solubility at neutral pH. Acidic or alkaline pH causes a change in protein conformation (denaturation), thereby reducing protein solubility. Protein solubility is influenced by amino acid composition, molecular weight, protein conformation, the balance between polar and non-polar groups in amino acids, and the solvent's pH (Awwali, 2017). The effect of extraction time on the protein content of soluble annatto extract is shown in Fig. 6.

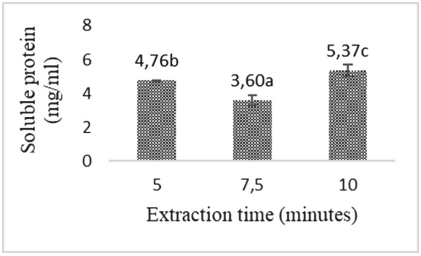

Fig. 6. The soluble protein of annatto extract in the extraction times variation.

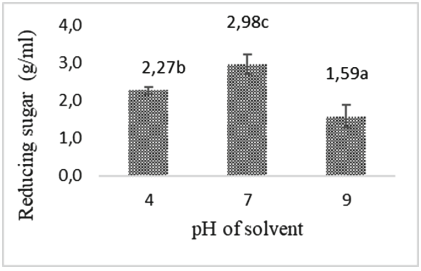

Fig. 7. The reducing sugar of annatto extract in the solvents of pH variation.

Figure 6 shows that the more prolonged the extraction time, the higher the soluble protein. The higher soluble protein is due to the more contact between the solvent and the dissolved protein, and the presence of stirring causes an increase in the dissolved protein content. This result is in line with Kurniati (2009), who states that the longer the extraction time, the more solute is taken in the solvent.

3.4 Reducing Sugar

Reducing sugars are sugars whose ends contain free aldehyde or ketone groups so that they can reduce electron-accepting compounds (Afriza et al., 2019) (Kunz 2011). The reducing sugar content of annatto extract at various solvent pH is shown in Fig. 7.

The highest reducing sugar was produced in distilled water solvent pH seven and the most. Neutral pH distilled water can extract reducing sugars better than acidic or basic pH. The lowest reducing sugar content in distilled water at pH nine is thought to be because the reducing sugar reacts with calcium which is used to adjust the pH. In alkaline solutions, reducing sugars can reduce metals. The sugar component is oxidized to aldonic, ketonic, or uronic (Indahyanti et al., 2014). Changes in the reduced sugar content of annatto extract were also affected by the extraction time (Fig. 8).

Extraction for 10 min produces the highest reducing sugar, while extraction for 7.5 min produces the lowest reducing sugar. Allegedly during the extraction reaction occurs changes in complex and simple carbohydrates. Extraction in a long time (10 min) is thought to be the hydrolysis of complex carbohydrates into sugars. During the reaction, it is suspected that there was a reaction between the reducing sugar and the metals or

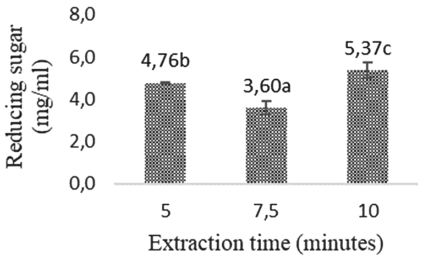

Fig. 8. The reducing sugar of annatto extract in the extraction times variation.

minerals present in the annatto extract, which caused a decrease in the reduced sugar. Gupta (2016) stated that annatto seeds contain sucrose. Sucrose is a non-reducing sugar that can be hydrolyzed into reducing sugars. Factors that affect hydrolysis include pH, temperature, and reaction time. The annatto extraction carried out at 80 °C was thought to cause sucrose to undergo hydrolysis. The process of sucrose hydrolysis in annatto is thought to occur after 7.5 min of extraction, which causes an increase in reduced sugar content.

4 Conclusion

Extraction of annatto using distilled water pH 7 resulted in turbidity, total solids, dissolved protein, and high reducing sugar. Annatto extraction for 10 min resulted in total dissolved protein and reduced sugars. Aquadest can be used as a solvent for chemical compounds found in annatto.

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Authors' Contributions. All authors contributed to this research.

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