# Evaluation of colour and physicochemical properties of annatto seed aquadest extract in the variation pH of solvent

*by* Susanto Sulistyo

**Submission date:** 31-Mar-2023 06:25AM (UTC+0700)
**Submission ID:** 2051461367
**File name:** annatto_seed_aquadest_extract_in_the_variation_pH_of_solvent.pdf (590.37K)
**Word count:** 4117
**Character count:** 20919

# Evaluation of colour and physicochemical properties of annatto seed aquadest extract in the variation pH of solvent

Cite as: AIP Conference Proceedings **2586**, 060009 (2023); https://doi.org/10.1063/5.0108054
Published Online: 24 January 2023

**Isti Handayani, Aisah Tri Septiana, Pepita Haryanti, et al.**

# Evaluation of Colour and Physicochemical Properties of Annatto Seed Aquadest Extract in the Variation pH of Solvent

Isti Handayani [1, a)], Aisah Tri Septiana[1,b)], Pepita Haryanti[1,c)], Budi Sustriawan[1,d)] and Susanto Budi Sulistyo[2,e)]

[1]*Department of Food Technology, Faculty of Agriculture, Universitas Jenderal Soedirman Jl. Dr.Soeparno, Karangwangkal, Purwokerto,53123, Indonesia*
[2]*Department of Agricultural Engineering, Faculty of Agriculture, Universitas Jenderal Soedirman. Jl. Dr.Soeparno, Karangwangkal, Purwokerto,53123, Indonesia*

[a)] Corresponding author: isti.handayani@unsoed.ac.id
[b)] aisyah.septiana@unsoed.ac.id
[c)] pepita'haryanti@unsoed.ac.id
[d)] budi.sustriawan@unsoed.ac.id
[e)] susanto.sulistyo@unsoed.ac.id

**Abstract.** *Bixa orellana* seeds (Annatto) have been used in food coloring and cosmetics. The main commercial processes to extract the pigment from annatto seeds were direct extraction into oil or aqueous alkali and indirect extraction using organic solvents. This research was held to determine the color and physicochemical properties of annatto extracts using aqua dest as a solvent. The extraction was carried out by maceration at various pH of aqua dest (4, 7, and 9) at 80ºC for 10 minutes. Color property of brightness (L), redness (a), and yellowness (b) was determined using a color reader. Physicochemical characterization was conducted by observing pH value, total titrated acid, total dissolved solids, and total solids. The results showed that the color and physicochemical properties of annatto extract were influenced by the pH of aqua dest influences. The highest brightness (43.8), redness (9.37), yellowness (42.5), and total soluble solid (1.67º Brix) was produced by extraction using pH 7 of aqua dest, while the highest of total titrated acid (4.2%) of extract produced in pH 4. The lowest pH of the extract (4.83) was resulted in extraction using aqua dest pH 4. The higher the pH of the solvent, the higher the pH of the extract. The different pH of aqua dest used to extract annatto seeds resulted in various color intensities and physicochemical property.

## INTRODUCTION

Public awareness to choose food that looks attractive and safe is increasing. Color is one of the attributes of food quality. Color can be used to attract consumers to choose and consume a food product. Colors or pigments can be obtained from the extraction of foodstuffs such as vegetables and fruit. Essential compounds in natural dyes are polyphenols, carotenoids, chlorophylls, and betalains [1]. One type of plant that produces carotenoid pigments is kesumba (*Bixa orrellana*. L). Kesumba is one of the carotenoid-producing plants called annatto. Carotenoid pigment in annatto consist of bixin and norbixin [2]. Bixin is non-polar, while norbixin is polar. Other pigments in annatto are carotene, cryptoxanthin, lutein, zeaxanthin,and methyl bixin[3].

Since people are now more concern about their health, they prefer to use natural food additives such as natural food colorant[4]. The pigments produced by the kesumba plant can be used as natural dyes that are safe and get recommendations from the FDA [4]. Annato pigment has a high tinctorial value and an outer color range comprising red, orange, and yellow hues [5]. Pigment analysis can be carried out by various methods, including using a color

*The Third International Symposium on Food and Agrobiodiversity (ISFA 2021)*
AIP Conf. Proc. 2586, 060009-1–060009-7; https://doi.org/10.1063/5.0108054
Published by AIP Publishing. 978-0-7354-4231-3/$30.00

reader. Identified pigments of seawedss based on their spectral and chromatographic properties and also confirmed by electrospray ionization-mass spectrometry (ESI-MS/MS) analysis [6]. Bixin (non-polar) is more soluble in vegetable oil; conversely, norbixin (polar) is more soluble in an aqueous solution. Norbixin is a dark red-brown to red-purple orange-yellow carotenoid [7], while bixin is dark red. As a colorant, annatto is used in cheeses, sausages, meat, and candies industries [8].

Some methods have been used to extract the annatto pigment, such as soxhlet extraction, maceration, vegetable oil extraction, alkaline solution, organic solvent extraction, supercritical $CO_2$, and ball milling grinder [8][9]. Most studies on annatto pigment in Indonesia are focused on the isolation and stability characterizations of bixin, while the norbixin potency as natural food colorant has not been developed yet. Norbixin is a dark red-brown to red-purple orange-yellow carotenoid derived from the outer seed coats of annatto.

Certain types of solvents for extraction are known toxicity and environmental hazard (e.g., benzene, chlorocarbons) and have not been permitted to be used in the manufacture of pharmaceuticals [10]. Performed annatto extraction using ethanol[11], while [12] performed annatto extraction using acetone. Annatto extraction using water (distillate water) is one of the profitable annatto extraction alternatives because water is a solvent that is easily obtained and the price is low. Aquadest as a solvent also has a high dielectric constant and polarity[13]. Rosmah performed annatto extraction using water[14]. The research results by [14] showed that the intensity of the annatto color was affected by the extraction temperature. Factors that influence the extraction process include the temperature of the extraction process [15][16], stirring speed, as well as solvent pH and extraction time [16]. The pH of the solvent was thought to affect the physicochemical characteristics of the annatto extract, which was caused by changes in the solubility of carotenoids and other components.The study of annatto extraction using waterat varying the pH of the solvent on the color and chemical characteristics of the annatto extract has not been carried out.

## MATERIAL AND METHODS

### Annato Extraction

Annato extraction was carried out by studies conducted by [14] and [17]. 25 gof annatto seeds was added to 90 mL of distilled water as the solvent. Maceration was performed through a magnetic stirrer at various heating temperatures of 70, 80, and 90 $^{o}$C for 30 minutes using aquadest (pH 7) as the solvent

### Method Color Measurement of Extract

Color Reader CR-10 (Konica Minolta Sensing, Inc, Japan) was used to measure the color of extract. The measurement method used is the absolute color system measurement L*, a*, and b*. The L* value indicates a change in brightness or lightness with a range of values from 0 (black) to 100 (white). The a* value indicates red- green color. The value of +a* from 0 to 100 for red, and –a from 0 to –80 for green. Meanwhile, the b* value represents the blue-yellow mixed chromatic color with a +b* value from 0 to +70 for blue and a –b* value from 0 to 70 for yellow. Samples that have been treated are placed under the camera. Furthermore, measurments are directed at the samples and the result of L*a*b* values will appear on the display screen [18].

### The pH of Extracts

The pH meter HANNA was used to measure the pH of the extract. The instruments were calibrated with buffer solutions pH 4 and 7 before use. Sample measurement was carried out by taking a sample of 5 ml, and then the electrode was rinsed with distilled water. The electrodes were dried with the tissue is then dipped in the sample. The measured pH is read as the pH value indicator has stabilized.

### Total Titratable Acid

The total titratable acid is carried out by calculating the acid equivalent of citric by titration method [19]. Samples were taken 10 ml for titration. Before the sample is titrated with two drops of 1% phenolphthalein (PP), the sample is titrated with 0.1 N NaOH until a pink color appears constant. The formula calculates acid content:

$$\text{Acid content (\%)} = \frac{V1 \times N \times B \times 100\%}{V2 \times 1000} \quad (1)$$

$V_1$ : Volume of NaOH (ml)
$V_2$ : Sample volume (ml)
N : Normality of NaOH (0.1 N)
B : Lactic Acid Molecular Weight (90)

### Total Soluble Solids

Measurement of total dissolved solids using a refractometer according to SNI 01-3546-2004. The total dissolved solids content of the sample was determined using a digital handheld refractometer (Model: PAL-1, Atago co, Ltd, Tokyo, Japan) at 25°C and calibrated using distilled water, as many as 1-2 samples were inserted into the prism refractometer, and the amount of dissolved solids content is expressed as °Brix [20].

## RESULT AND DISCUSSION

### Color Characterization of Annatto Extracts

The major pigments of annatto are bixin and non-bixin9. Bixin (non-polar) is more soluble in vegetable oil. On the other hand, norbixin (polar) is more soluble in an aqueous solution. Bixin and norbixin has two stereoisomer configuration, i.e cis and trans.

Analysis of the color of the annatto extract was carried out using the Color Reader tool. The tool measures the material's color with three values symbolized by the letters L, a, and b. The L value represents the degree of brightness of the product, the a value represents the color gradation from green to red, while the b value represents the color gradation from blue to yellow. For values of a and b, the range of values is negative to positive.

*Brightness level (L Value)*

The results showed that the pH of the solvent had a significant effect on the brightness of the extract. Variations in solvent pH have a significant effect on the brightness of the extract (Figure 1).

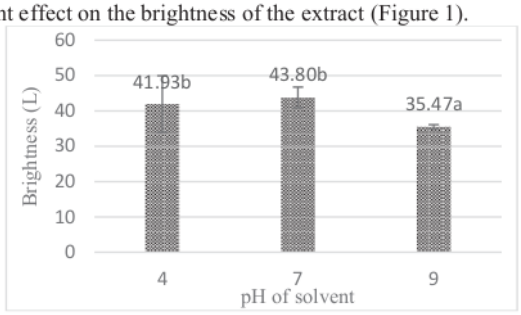


**FIGURE 1**. The brightness of annatto extract in the variation of pH of the solvent

In Figure 1, the lowest brightness (35.47) was produced in extraction using aquaest of pH 9. Extraction using pH 7 of aqua dest produced the highest brightness and was not significantly different from pH 4. The main carotenoid extracted at aquadest pH 7 was presumed to be polar norbixin. Norbixin is a polar carotenoid, so it has a high solubility in aqua dest. The aqua dest pH 7 can extract norbixin which is higher than pH 4 and 9. The difference in the ability to extract pigment is thought to be caused by differences in pH, which cause differences in the dielectric constant of water. Water is the most polar solvent with a dielectric constant of 80. According to [21], the variation in pH at water solvent used, the polarity water decreases so that it can extract polar compounds and semi compounds polar. pH 9 of solvent was adjusted with $Ca(OH)_2$, resulting in a more non-polar solvent, while pH 4 was modified with polar citric acid. Decreasing polarity of the water is thought to cause the non- polar bixin to be extracted from the distilled water.

Bixin is non-polar, giving a dark red color [22], so a pH of 9 aqua dest results in a decreased brightness (darker). According to [23], the higher the pH of the water, the lower its polarity. The greater the value of L, the color of the kesumba seed extract will be brighter, while the smaller the value of L, the color of the extract will be darker [28-30].

The alkaline pH of the solvent will result in the color of the coriander seed extract, which tends to be more concentrated or darker. The annatto seed extract that uses a solvent of pH 4 and pH 7 has not been extracted completely

*Redness ($a^+$value)*

The a value consists of a+ (red) and a- (yellow). The color of the annatto extract at the pH variation of the solvent is shown in Figure 2.

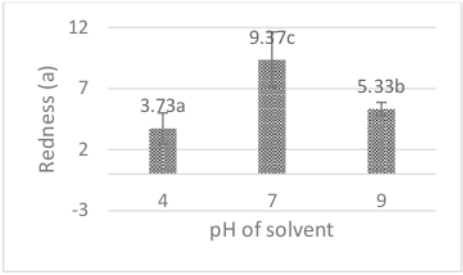


**FIGURE 2.** The redness of annatto extract in the variation of pH of the solvent.

The results showed that variation in the pH of the solvent had a significant effect on the intensity of the red color of the annatto extract. Based on Figure 2, the highest redness value (9.37) is produced in pH 7, while the lowest redness value (3.73) is produced in pH 4. The main carotenoid extracted at aquadest pH 7 was presumed to be polar norbixin The difference in the pH of the solvent causes differences in the dielectric constant so that it causes differences in the ability to extract the pigment. Aquades pH 7 is thought to be more capable of removing the polar norbixin. The dielectric properties of the solvent used in the extraction process determine its ability to extract nutrients from a selected plant matrix [24].

The higher color intensity at pH 7solvent is also thought to be due to the higher stability of the pigment at neutral pH. The pH of the solvent at the time of extraction can change the stability of the carotenoid pigment. The effect of pH on carotenoid pigments from alkesa fruit resulted in carotenoid pigments in alkesa extract being more stable at neutral pH (pH 7) storage compared to storage at acidic pH (pH 4) and alkaline pH. Carotenoids are pigments with properties that are easy to isomerizein acidic or alkaline conditions. The reddish value of the annatto extract at pH 4, and pH 9 solvents to tend to have a lower reddish value. Another thing can be caused by the content of bixin which gives a red color in alkaline conditions can be converted into norbixin compounds that give a yellow color. Bixin can be converted into norbixin by adding an alkaline solution [8].

*Yelloness (b+ value)*

The value of b consists of b+ (yellow) and b- (blue). The color of the annatto extract at the pH variation of the solvent is shown in Figure 3.

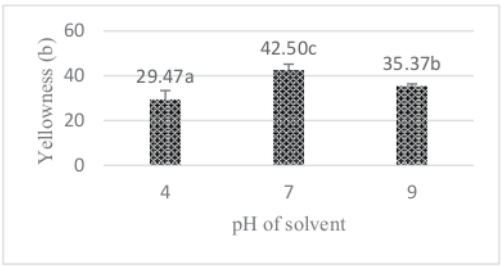


**FIGURE 3.** The yellowess of annatto extract in the variation of pH of the solvent.

Based on Figure 3, the highest yellowness value (42.50) is produced in pH 7, while the lowest (29.47) is produced in pH 4. The carotenoids norbixin and bixin are composed of red and yellow components with different intensities. According to [25] annatto extraction using ethanol (polar) produced an orange color consisting of red with an intensity of 12 and yellow with an intensity of 50. It was suspected that at acidic and alkaline pH, norbixin was degraded. The results of this study are in line with [7] which stated that the absorbance of norbixin was smaller at pH 4 compared to pH 7. The double bonds in carotenoids caused these compounds to be easily degraded by pH [2].

## Physicochemical characterization of annatto extracts

[9] stated that the type of solvent and the ionic strength of the solvent affect the effectiveness ofthe extraction of bioactive components. In this study, the effect of the pH of the aqua dest solvent on the physical and chemical properties of the annatto extract included the pH of the extract, the total titrated acid, total dissolved solids, and total solids.

*The pH of extract*

Measurement of pH value is one of the parameters to determine changes in the acidity level. The pH of the solvent has significantly affected the pH of the extract (Figure 4).

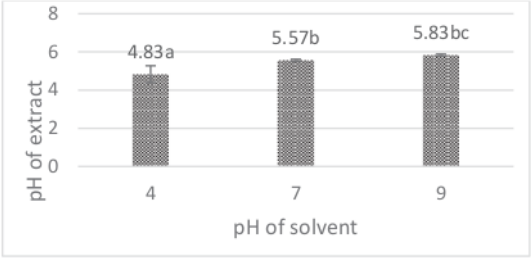


**Figure 4.** The pH of annatto extract in the variation of pH of the solvent.

Figure 4 shows an increase in the pH of the solvent resulting in a higher pH. The setting of pH 4 aaudest is done by adding citric acid, while the setting of the solvent for pH 7 is done by adding $Ca(OH)_2$. The addition of citric acid into distilled water causes the pH of the resulting extract to be low due to the addition of organic acids from the added citric acid. The addition of Ca $(OH)_2$ is thought to cause the formation of salts as a result of the reaction between Ca $(OH)_2$ and organic acids found in annato extract. The annatto extract seed oil contains essential oils in the form of farnezyl acetate (11.6%), occidentalol acetate (9.7%), ishwarane (9.1%) [26]. The addition of $Ca(OH)_2$ is thought to cause a reaction with these components thereby increasing the pH of the extract.

*Total titratable acidity (TTA)*

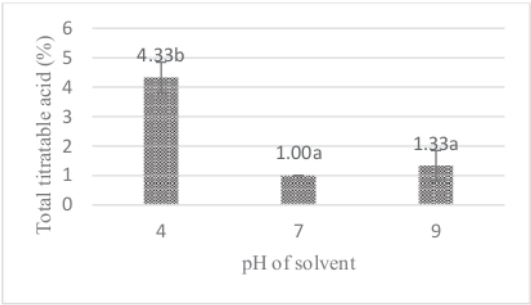


**FIGURE 5.** The total titratable acid of annatto extract in the variation of pH of the solvent.

The titrated total acid is a test used to measure the concentration of total acid in food. Sour is measured in the general TAT test organic acids such as citric, malic, lactate, and tartarate. The total titratable acidity of the annatto extract at the pH variation of the solvent is shown in Figure 5. Based on Figure 5, the highest of total titrated acid (4.33%) was produced at pH 4 solvent. This was due to the adjustment of pH 4 with the addition of citric acid, an organic acid. So that the addition of citric acid causes an increase in the total titrated acid.

*Total soluble solids*

The measured components in total dissolved solids are total sugar, pigment, organic acids, and protein content [27]. The results showed that the variation in the pH of the solvent had no significant effect onthe total soluble solid of the annatto extract. Annatto extracts total dissolved solids content at pH 4, 7 and 9 were 1.33; 1.67 and 1 mg/mL. There is a tendency for total dissolved solids at pH 7 to be higher than pH 4 and 9. Extraction at neutral pH has the highest level of polarity to extract sugar compounds, pigments, organic acids,proteins, and polar pigments.

## CONCLUSION

Aquadest can be used as an alternative solvent to extract the annatto pigment. Extraction using aquadest by maceration is considered profitable because it is easy to obtain, the price is cheap. The use of aqua dest pH 7 produces a higher color intensity (brightness, redness and yellowness) than pH 4 and 9, but while pH 4 of aquadest produce hgher in total titratable acid. The different pH of aqua dest used to extract annatto seeds resulted in various color intensities and physicochemical property.

## ACKNOWLEDGMENTS

The authors acknowledge to the financial support from Jenderal Soedirman University, through Riset Dasar 2021.

## REFERENCES

1. O. O Awolu and O. A. Oladeji, Natural slant pigments and derivatives in functional foods developments, Eurasian J. Food Sci. Technol **5**, 25–40 (2021).
2. C. Gallardo-Cabrera and A. Rojas-Barahona, Stability study of an aqueous formulation of the annatto dye, Int. Food Res. J **22**, 2149–2154 (2015).
3. M. J. Scotter., L. A. Wilson., G. P. Appleton, and L. Castle, Analysis of annatto (*Bixa orellana*) food coloring formulations. 2. determination of aromatic hydrocarbon thermal degradation products by gas chromatography, J. Agric. Food Chem **48**, 484–488 (2000).
4. N. Aryanti and A Nafiunisa, Extraction, characterization and degradation of chlorophyll from Suji Leaves (*Pleomele angustifolia*), Orient. J. Chem **33**, 3185–3190 (2017).
5. N. N. Husa., F. Hamzah, and H. M. Said, "Characterization and storage stability study of bixin extracted from *Bixa orellana* using organic solvent", in *IOP Conference Series: Material Science and Engineering*, edited by P. Mésároš *et al.* (IOP Publishing, Bristol 2018), pp. 1–7.
6. Heriyanto, A. D. Juliadiningtyas., Y. Shioi., L. Limantara, and T. H. P. Brotosudarmo, Analysis of pigment composition of brown seaweeds collected from Panjang Island, Central Java, Indonesia, Philipp. J. Sci **146**, 323–330 (2017).
7. S. Suparmi and L. Limantara, "Stability characterization of norbixin pigment from kesumba keling ( *Bixa orellana* L", in *8th National Student Conference "Smart Food: Lifestyle and Health Reconciled",* (Universitas Khatolik Soegijapranata, Semarang, 2008).
8. G. F. Silva., F. M. C. Gamarra., A. L. Oliveira, and F. A. Cabral, Extraction of bixin from annatto seeds using supercritical carbon dioxide, Brazilian J. Chem. Eng **25**, 419–426 (2008).
9. T. V. Cuong and K. B. Chin, Effects of annatto (*Bixa orellana* L.) seeds powder on physicochemical properties, antioxidant and antimicrobial activities of pork patties during refrigerated storage, Korean J. Food Sci. Anim. Resour **36**, 476–486 (2016).
10. A. C. Kumoro., M. Hasan, and H. Singh, Effects of solvent properties on the soxhlet extraction of diterpenoid lactones from Andrographis paniculata leaves, ScienceAsia **35**, 306–309 (2009).

11. A. Venugopalan and P. Giridhar. Bacterial growth inhibition potential of annatto plant parts, Asian Pac. J. Trop. Biomed **2**, 879–882 (2012).
12. M. Yolmeh., M. B. Habibi-Najafi., R. Farhoosh, and F. Salehi, Modeling of antibacterial activity of annatto dye on Escherichia coli in mayonnaise, Food Biosci **8**, 8–13 (2014).
13. M. E. Yulianto., P. Kusumo., I. Hartati, and A. Wahyuningsih, Esubcritical water extraction of gingerol from zingiber officinale, Rasayan J. Chem **10**, 738–743 (2017).
14. E. Rosamah., R. Ramadan, and I. W. Kusuma, "Stabilitas warna biji tumbuhan annatto ( *Bixa orellana* L .) sebagai bahan pewarna alami", in *Prosiding Seminar Nasional Masyarakat Peneliti Kayu Indonesia (Mapeki) XVI*, edited by W. Suwinarti *et al.* (MaPeKi, Bogor, 2014), pp. 209–214.
15. M. Yuniwati., A. W. Kusuma, and F. Yunanto, "Optimasi kondisi proses ekstraksi zat pewarna dalam daun suji dengan pelarut etanol", in *Prosiding Seminar Nasional Aplikasi Sains & Teknologi (SNAST) Periode III*, (IST AKPRIND, Yogyakarta, 2012), pp. 257–263.
16. Paryanto., Hermiyanto, and S. D. S. Sanjaya. Pembuatan zat warna alami dari biji kesumba dalam bentuk konsentrat tinggi untuk pewarna makanan, J. Ekuilibrium **13**, 55–58 (2014).
17. M. Abayomi., A. S. Adebayo., D. Bennett., R. Porter, and J. Shelly-Campbell, In vitro antioxidant activity of *Bixa orellana* (*Annatto*) seed extract, J. Appl. Pharm. Sci **4**, 101–106 (2014).
18. A. I. N. Tari., C. B. Handayani, and S. Sudarmi, Potensi probiotik indigenus Lactobacillus plantarum Dad 13 pada yogurt dengan suplementasi ekstrak ubi jalar ungu untuk penurun diare dan radikal bebas, J. Agritech **36**, 7–14 (2016).
19. M. D. Hariyanti., Y. B. Pramono, and S. Mulyani, Total asam, viskositas, dan kesukaan pada yogurt drink dengan sari buah mangga (*Mangifera indica*) sebagai perisa alami, J. Apl. Teknol. Pangan **2**, 40–43 (2013).
20. N. Ismawati, Nilai Ph, total padatan terlarut, dan sifat sensoris yoghurt dengan penambahan ekstrak bit (*Beta vulgaris* L.), J. Apl. Teknol. Pangan **5**, 89–93 (2016).
21. B. C. B. S. Mello., P. M. Kakuda, and M. D. Hubinger, "Influence of pH variation during propolis extraction with the use of water as solvent" In *Proceedings of the 11th International Congress on Engineering and Food (ICEF11)*, edited by P. S. Taoukis *et al*. (Cosmosware, Athens, 2011), pp. 87–96.
22. D. Raddatz-Mota., L. J. Pérez-Flores., F. Carrari., M. Insani., R. Asis., J. A. Mendoza-Espinoza., F. Díaz de León-Sánchez., and F. Rivera-Cabrera, Chemical characterization and quantification of the pigment extraction yield of seven Mexican accessions of *Bixa orellana*, Rev. Mex. Ing. Quim **15**, 727–740 (2016).
23. S. N. Rismawati and I. Ismiyati, Pengaruh variasi pH terhadap kadar flavonoid pada ekstraksi propolis dan karakteristiknya sebagai antimikroba, J. Konversi **6**, 89–94 (2017).
24. A. Singh., G. R. Nair., P. Liplap., Y. Gariepy., V. Orsat, and V. Raghavan, Effect of dielectric properties of a solvent-water mixture used in microwave-assisted extraction of antioxidants from potato peels, Antioxidants **3**, 99–113 (2014).
25. I. Handayani and R. Setyawati, "Evaluation types of solvents on the extraction of *Bixa orellana* and application of extract on a chicken sausage product as natural colour and antioxidant sources", in IOP Conference Series: Earth Environmental Science, edited by B. Nurhadi *et al*. (IOP Publishing, Bandung, 2020), pp. 1–9.
26. Shahid-ul-Islam, L. J. Rather, and F. Mohammad, Phytochemistry, biological activities and potential of annatto in natural colorant production for industrial applications, A review. J. Adv. Res **7**, 499–514 (2016).
27. I. M. Merdana., I. K. Suada, and I. P. A. S, Reducing total dissolved solid of livestock wastewater with moringa seed powder, Adv. Trop. Biodivers. Environ. Sci **4**, 1–4 (2020).
28. Suminar, A.T., Al-Baarri, A.N., Legowo, A.M. Potravinarstvo Slovak Journal of Food Sciences, 11 (1), pp. 417-424. (2017)
29. Al-Baarri, A.N., Legowo, A.M., Widayat. IOP Conference Series: Earth and Environmental Science, 102 (1), art. no. 012003 (2018)
30. Al-Baarri, A.N., Widayat, Legowo, A.M., Ranini, A.A., Setyawan, B.A., Lestari, F.P. IOP Conference Series: Earth and Environmental Science, 653 (1), art. no. 012052, (2021)

# Evaluation of colour and physicochemical properties of annatto seed aquadest extract in the variation pH of solvent

ORIGINALITY REPORT

| 12% | 6% | 9% | 2% |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

4%

★ I Handayani, R Setyawati. "Evaluation types of solvents on the extraction of Bixa orellana and application of extract on a chicken sausage product as natural colour and antioxidant sources", IOP Conference Series: Earth and Environmental Science, 2020

Publication

| | | | |
|---|---|---|---|
| Exclude quotes | On | Exclude matches | < 2% |
| Exclude bibliography | On | | |