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The color characteristics of annatto from achiote seeds (Bixa orellana L.) extracted with the assistance of microwaves.

[Karakteristik warna annatto dari Bixa orellana L. yang diekstraksi dengan metode ekstraksi berbantuan gelombang mikro]

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ABSTRACT

The extraction method affects the functional properties of the extract. This study aimed to analyze the color characteristics of annatto produced using maceration supported by microwave-assisted extraction (MAE) using aquadest pH 4, 7, and 9. Maceration was performed at 80°C for 5 minutes. Extraction supported by MAE was conducted for 2, 4, and 6 minutes at a power of 100 W. The observed parameters included bixin, norbixin, and color. Bixin and norbixin were analyzed to determine the pigment. Color was measured using a colorimeter to assess L (brightness), a (redness), and b* (yellowness), and the maximum wavelength was determined. The research was conducted using a Completely Randomized Factorial Design. The control used only maceration with distilled water without pH adjustment. The results showed that extraction using the maceration or maceration-supported MAE methods produced 1.5% pigment. Higher brightness was achieved by the maceration supported by the MAE method using the acid solution for 2 minutes. A higher intensity of red color was created by the maceration supported by the MAE method with an alkaline solution for 4 minutes, while there was no difference in the intensity of yellow color between maceration supported by MAE and simple maceration with distilled water (control). Distilled water as a solvent effectively extracts annatto using MAE-assisted maceration and simple maceration (control). Annatto extracts obtained from both methods exhibited maximum absorption at 300 nm.*

Keywords: annatto, color, extraction, maceration, microwave-assisted extraction

ABSTRAK

Metode ekstraksi mempengaruhi sifat fungsional ekstrak. Penelitian ini bertujuan mengevaluasi karakteristik warna annatto yang diperoleh menggunakan metode maserasi berbantu microwave assisted extraction (*MAE*) menggunakan pelarut aquades pH 4,7 dan 9. Maserasi dilakukan pada suhu 80°C selama 5 menit. Ekstraksi berbantu *MAE* dilakukan selama 2, 4 dan 6 menit dengan daya 100 W. Parameter pengamatan meliputi bixin, nor bixin dan warna. Bixin dan norbixin dianalisis untuk mengetahui kadar pigmen ekstrak. Warna diukur menggunakan *color reader* untuk menentukan kecerahan (L), kemerahan (a*) dan kekuningan (b*) serta ditentukan panjang gelombang maksimum. Desain penelitian menggunakan Rancangan Acak Lengkap Faktorial, sebagai kontrol ekstraksi hanya dilakukan secara maserasi. Hasil penelitian menunjukkan ekstraksi dengan metode maserasi maupun maserasi berbantu *MAE* menghasilkan pigmen sebesar 1,5%. Kecerahan yang lebih tinggi dihasilkan pada metode maserasi berbantu *MAE* menggunakan larutan asam selama 2 menit. Intensitas warna merah yang lebih tinggi dihasilkan pada metode maserasi berbantu *MAE* menggunakan aquades pH 9 selama 4 menit, namun intensitas warna kuning yang dihasilkan maserasi berbantu *MAE* maupun maserasi (kontrol) tidak menunjukkan perbedaan. Penggunaan aquades sebagai pelarut efektif untuk ekstraksi annatto baik secara maserasi berbantu *MAE* maupun maserasi (kontrol). Ekstraksi menggunakan aquades memiliki serapan maksimal pada panjang gelombang (λ) 300 nm.

Kata kunci: annatto, ekstraksi, maserasi, microwave-assisted extraction, warna

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Introduction

Annatto is a natural pigment that is safe for food and has been approved by the *Food and Drug Administration (FDA)*. Annatto is obtained from the seeds of the achiote plant (*Bixa orellana* L.). Annatto extract has a high coloring strength, making it useful in the food and cosmetic industries. The active compounds responsible for the pigment's color are carotenoids (Witono et al., 2022). Achiote seeds contain approximately 7% annatto pigment (Bindyalaxmi et al., 2022). The primary pigment in annatto is bixin, which exists in ester form and belongs to the carotenoid group (Finkler et al., 2024). Bixin makes up about 80% of the total pigment content in annatto (Finkler et al., 2024). Another pigment found in annatto is norbixin, the concentration of which depends on the ripeness of the achiote (Aluko, 2024). Bixin is nonpolar, while norbixin is polar (Nurtiana et al., 2023); Handayani et al., 2024). Carotenoid pigments are advantageous because they provide a range of colors from yellow to red (Maleta et al., 2018), depending on pH and the type of solvent used (Anggreini, 2019). Using an acidic solution (distilled water at pH 4) results in a reddish-yellow hue with higher brightness compared to an alkaline solution (distilled water at pH 9) (Handayani et al., 2021). Solution produces a reddish-yellow annatto extract, while acetone produces an orange-colored extract (Nurtiana et al., 2023). The main drawback of annatto as a natural pigment is its susceptibility to degradation due to high temperatures, light, and oxygen exposure (Chuyen & Eun, 2021). Bioactive compounds can be extracted using both conventional and non-conventional methods. Conventional extraction methods include maceration, Soxhlet extraction, infusion, and percolation (Bitwell et al., 2023). However, conventional methods require longer processing times, more significant amounts of solvents, and higher degradation of bioactive compounds compared to non-conventional methods (Bitwell et al., 2023). Some non-conventional methods for extracting annatto seeds include spouted bed drying, sonication, and microwaves (Quiroz et al., 2019). The advantages of non-conventional methods include shorter processing times, reduced solvent use, lower bioactive compound degradation, higher energy efficiency, and increased yield (Bitwell et al., 2023).

Several non-conventional methods used to extract annatto include oil solvent extraction (Mehta et al., 2015); the use of CO_2, ethanol, and ethanol-water mixtures (Aluko, 2024); Soxhlet extraction with ethyl acetate solvent (Sabuz et al., 2020); the use of chloroform (Franklin et al., 2023); the use of acetone (Pattanaik et al., 2018); and maceration extraction using distilled water with pH adjustment (Handayani et al., 2024). Organic solvents used for extraction may leave toxic residues that pose health risks (Taham et al., 2015). Handayani et al., 2021 stated that distilled water with pH adjustments to 4 (acidic solution) and 9 (alkaline solution) is a safe alternative for annatto extraction via maceration.

Microwaves can also be a non-conventional extraction method, including *Microwave-Assisted Extraction (MAE). MAE* is an extraction technique that utilizes microwave energy. This technology is suitable for extracting thermolabile compounds because it provides better temperature control than conventional heating processes. The characteristics of the extract obtained using the *MAE* method are influenced by factors such as extraction time, temperature, and the solvent used (Kristanti et al., 2019). The polarity of the extracted material determines the suitability of a solvent. The pH adjustment of distilled water alters its dielectric constant, thereby changing its polarity (Rismawati & Ismiyati, 2017). Optimization of extraction conditions to maximize bixin content has been carried out using *MAE* at 700 W power, pH 4 for 5 minutes, with 95% ethanol as the solvent, applying a response surface design (Quiroz et al., 2019). The color characteristics of annatto extract obtained through maceration and *MAE* require further development. In this study, maceration assisted by *MAE* was tested to evaluate the characteristics of annatto extract by adjusting the pH of the distilled water solvent and extraction time. Maceration assisted by abrasion using a magnetic stirrer was employed at the beginning of the maceration process because annatto pigments and compounds are located in the outer layer of the seeds, where they are strongly

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bound. Therefore, an abrasion-assisted extraction process is needed to release these components. Maceration using a magnetic stirrer is one approach to extracting annatto compounds. The *MAE*-assisted maceration method is expected to produce higher color intensity than a single extraction method. This study aims to evaluate the color characteristics of annatto extract obtained using *MAE*-assisted maceration with distilled water as the solvent, adjusted for pH and extraction time. The novelty of this research lies in the use of *MAE*-assisted maceration for annatto seed extraction using distilled water, focusing on the resulting color characteristics.

Material and method

Materials and equipment

The primary material used in this study was annatto seeds obtained from Ledug Village, Banyumas Regency. Other materials included citric acid (Merck), $Ca(OH)_2$ (Merck), tetrahydrofuran (Merck), acetone (Merck), and KOH (Merck). The equipment used in this study included a pH meter (Hanna Instrument), microwave (Sharp R751GX), UV-Vis spectrophotometer (Shimadzu 2UV-1800), color reader (Konica Minolta CR-10, Japan), and magnetic stirrer (Capp 15Rondo CRS-22H, Germany).

Research method

This study employed a factorial, Completely Randomized Design with two tested factors: pH of distilled water solvent, consisting of three levels: pH 4, pH 7, and pH 9; Extraction time using *Microwave-Assisted Extraction (MAE),* consisting of three levels: 2 minutes, 4 minutes, and 6 minutes. The experiment was conducted with three replications, and sample analysis was performed in duplicate.

The research data were analyzed using a one-way analysis of variance (ANOVA). Further analysis was conducted using Duncan's Multiple Range Test if significant differences were found. The least significant difference (LSD) method was used for pairwise comparisons. Differences were considered statistically significant at $p < 0.05$. Data analysis results are presented as mean ± standard deviation.

Research implementation

Annatto extraction from achiote seeds

The extraction of annatto from achiote (*Bixa orellana*) seeds began with the preparation of distilled water as the solvent. The pH of distilled water was adjusted to 4, 7, and 9 using citric acid and $Ca(OH)_2$ (Handayani et al., 2021). Citric acid was used to achieve pH 4, while $Ca(OH)_2$ was used to adjust the pH to 7 and 9. A total of 25 g of achiote seeds was added to 90 mL of pH-adjusted distilled water. The extraction process began with maceration, where stirring was performed using a magnetic stirrer. Maceration was conducted at 80°C for 5 minutes. After maceration, each sample (achiote seeds in distilled water at pH 4, 7, and 9) was further processed using Microwave-Assisted Extraction (*MAE*) with a Sharp R751GX microwave at 100 W power for 2, 4, and 6 minutes, with the microwave temperature maintained between 90-100°C. As a control, extraction was performed only using maceration at 80°C for 5 minutes, with distilled water at its natural pH (6.6), without pH adjustment and *MAE* treatment. The crude extract obtained was filtered using filter paper, and the resulting filtrate was used for further analysis. The filtrate analysis included determining bixin and norbixin pigment content, measuring extract color (L, a*, and b* values), and determining maximum wavelength absorption.

Research parameters

(1) Bixin content analysis in filtrate

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Bixin content was measured using the Joint *FAO/WHO Expert Committee on Food Additives (JECFA)* method (Smith & Wallin, 2006). The procedure was as follows: 1 mL of annatto extract was mixed with 10 mL of tetrahydrofuran until fully dissolved, 1 mL of the resulting solution was then diluted with 100 mL of acetone, and the absorbance (A) of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 487 nm.

The bixin content was calculated using the following formula:

$$\% \text{ bixin} = \frac{\text{A x } 100.000}{3.090 \text{ x sample weight (mg)}} \text{ x } 100\%$$

A= absorbance of the sample at 487 nm

(2) Analysis of norbixin content in filtrate

Bixin content was determined using the Joint FAO/WHO Expert Committee on Food Additives method (Smith & Wallin, 2006). 1 mL of annatto extract was dissolved in 100 mL of 0.5% KOH solution. Then, 1 mL of this solution was taken and diluted in 100 mL of 0.5% KOH solution. The absorbance (A) of the solution was measured using a UV-Vis spectrophotometer (Shimadzu UV-1800) at a wavelength of λ 482 nm. The norbixin content is expressed using the following formula:

$$\% \text{ Norbixin} = \frac{\text{A x } 100.000}{3.870 \text{ x sample weight (mg)}} \text{ x } 100\%$$

A = absorbance of the sample at λ 482 nm.

(3) Filtrate color measurement

The color of the annatto extract was measured using a colorimeter, referring to (Pertiwi et al., 2022) with modifications. The annatto extract was dropped onto a 3 × 3 cm white paper. Then, the colorimeter was turned on, and the reading mode was set to L, a*, b*. L value represents lightness (brightness), the a* value indicates the green-red spectrum and the b* value indicates the blue-yellow spectrum.

(4) Measurement of maximum absorbance of filtrate

The maximum wavelength was measured using a spectrophotometer. A total of 10 mL of annatto extract was dissolved in 100 mL of distilled water (aquades), and its absorbance was measured at wavelengths 300, 350, 400, 450, and 500 nm.

Results and discussion

Bixin and norbixin content in filtrate

Bixin and norbixin belong to the carotenoid compound group, which plays a significant role in the coloration of annatto. Bixin is more soluble in nonpolar solvents, whereas norbixin is more soluble in polar solvents (Nurtiana et al., 2023). The bixin and norbixin content of annatto extract obtained using *Microwave-Assisted Extraction (MAE)* maceration with distilled water (aquades) as the solvent under different pH variations, and the control (maceration method) is presented in Table 1. The highest bixin content (1.07%) was obtained using distilled water at pH 9 with a 4-minute extraction time, which was not

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significantly different from the control. In contrast, other treatments resulted in lower bixin levels than the control. Bixin is a nonpolar compound, making it more soluble in distilled water at pH 9 than at pH 4 or 7. Adjusting the pH of distilled water alters its dielectric constant, thereby reducing polarity (Chong & Chua, 2020). Longer extraction time increases the amount of bixin extracted; however, heat may also lead to degradation (Cabrera & Barahona, 2015).

Table 1. Bixin and norbixin levels in annatto extract after filtration

Treatment		Bixin (%)	Norbixin (%)
Solvent pH	Extraction time (minutes)		
4	2	0.59 ± 0.46^{a}	0.46 ± 0.09^{c}
	4	0.54 ± 0.44^{a}	0.27 ± 0.13^{a}
	6	0.76 ± 0.60^{a}	0.35 ± 0.12^{a}
7	2	0.77 ± 0.47^{a}	0.53 ± 0.36^{c}
	4	0.65 ± 0.51^{a}	0.34 ± 0.20^{a}
	6	0.94 ± 0.71^{a}	0.39 ± 0.02^{ab}
9	2	0.74 ± 0.47^{a}	0.32 ± 0.11^{a}
	4	1.07 ± 0.69^{b}	0.45 ± 0.22^{c}
	6	0.92 ± 0.63^{a}	0.38 ± 0.08^{ab}
Control (pH 6.6)		1.07 ± 0.80^{b}	0.49 ± 0.23^{c}

Explanation: Numbers followed by the same superscript letter in the same column indicate no significant difference at the 5% level according to the DMRT test.

An increase in the pH of distilled water toward the alkaline range reduces its dielectric constant. Solvent polarity is expressed as the dielectric constant, and a lower dielectric constant makes the solvent more nonpolar (Dewi et al., 2020). Since bixin is nonpolar, it dissolves more readily in a solvent with a pH of 9. However, prolonged extraction at high temperatures can lead to bixin degradation. Therefore, using distilled water at pH 9 with a 4-minute extraction time resulted in a higher bixin content than pH 4 and 7. In contrast, extending the extraction time to 6 minutes at pH 9 decreased bixin content. This reduction was due to bixin degradation caused by prolonged exposure to heat (Cabrera & Barahona, 2015). In an alkaline pH, bixin is more soluble, leading to a higher pigment concentration.

The compounds in the material can be extracted well using a solvent with a suitable dielectric constant or polarity (Rismawati & Ismiyati, 2017). However, annatto begins to degrade at 70°C (Cabrera & Barahona, 2015). Optimization of annatto seed extraction using *MAE* with the Box-Behnken Experimental Design (BBD) was conducted to increase bixin content (Quiroz et al., 2019). The *MAE* method with 700 W power, pH 4 for 5 minutes, and 95% ethanol concentration resulted in a bixin yield of 0.33 ± 0.04%, whereas using pH 11 for 5 minutes and 73% ethanol concentration resulted in a bixin yield of 0.13 ± 0.02% (Quiroz et al., 2019). Extraction using maceration-assisted *MAE* with pH adjustment in this study yielded bixin levels ranging from 0.54% to 1.07%. Therefore, the maceration-assisted *MAE* method in this study produced a higher bixin content.

Annatto extraction using the maceration method without *MAE* resulted in a higher bixin yield than *maceration-assisted MAE*, except when using distilled water at pH 9. Therefore, the maceration-assisted *MAE* method is less effective for bixin extraction, even though the bixin yield was higher than in previous studies (Quiroz et al., 2019). In this study, maceration-assisted *MAE* using distilled water resulted in a lower bixin content. Several factors are suspected to contribute to the lower bixin yield, including the longer total extraction time in maceration-assisted *MAE* compared to maceration alone and the higher temperature generated by *MAE* power (ranging from 80°C to 100°C), depending on the extraction duration. The increase in extraction temperature leads to higher annatto degradation (Cabrera & Barahona, 2015).

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Norbixin has been added to various foods to provide a yellow-to-orange color. It is a carotenoid that ranges in color from dark reddish-brown to red-purple and orange-yellow. High levels of norbixin in maceration-assisted *MAE* were obtained using distilled water at pH 4 and 7 for 2 minutes, at pH 9 for 4 minutes, and in the control. Distilled water has the highest polarity at neutral pH, and changes in pH lead to variations in the dielectric constant, reducing polarity. Norbixin is polar, making it more soluble at pH 7 than at pH 4 and 9. Adjusting the pH of distilled water to 4 and 9 decreases its polarity. Extending the extraction time from 2 to 4 and 6 minutes increases the extraction temperature, accelerating norbixin degradation (Cabrera & Barahona, 2015). Changes in the pH of distilled water reduce its dielectric constant, leading to alterations in polarity (Dewi et al., 2020).

Norbixin and bixin are carotenoid compounds that can degrade due to processing conditions such as solvent type, extraction temperature, extraction time, and light exposure (Chuyen & Eun, 2021). Both bixin and norbixin begin to degrade at 70°C. Some degradation products of annatto pigments include m-xylene, toluene, and tuloic acid (Chuyen & Eun, 2021). High norbixin levels were also obtained in the control treatment, indicating that using *MAE* to continue maceration leads to bixin degradation unless *MAE* is performed for a short duration (2 minutes). A shorter *MAE* duration (2 minutes) results in a lower temperature (90°C). Annatto extracted using a 0.50% potassium hydroxide solution produced norbixin levels ranging from 0.95% to 1.69%, depending on the annatto variety used (Bindyalaxmi et al., 2022). orbixin levels obtained through maceration or maceration-assisted *MAE* using distilled water, with or without pH adjustment, were lower than 0.951% (Bindyalaxmi et al., 2022). The use of 80°C for 5 minutes during maceration, whether followed by *MAE* or not, is suspected to cause norbixin degradation, leading to a decrease in its concentration. The lower yield may also be due to differences in the annatto varieties used in the study, as different annatto varieties produce varying pigment levels (Bindyalaxmi et al., 2022).

The study results show that annatto extraction using the maceration method (control) and maceration-assisted *MAE* with distilled water as a solvent produced a high total pigment yield. The pigment content of 1.52%, consisting of 1.07±0.69% bixin and 0.45±0.22% norbixin, was obtained using maceration-assisted *MAE* with distilled water at pH 9 for 4 minutes. Meanwhile, the control method yielded a total pigment content of 1.56%, consisting of 1.07±0.80% bixin and 0.49±0.23% norbixin. Annatto-extracted acetone via Soxhlet extraction for 120 minutes yielded 44% bixin (Chuyen & Eun, 2021). Annatto extraction using a 0.5 M NaOH solution resulted in a norbixin concentration of 0.82 g/mL (Witono et al., 2022). The pigment content in annatto extract is influenced by several factors, including the extraction method and plant variety (Chuyen & Eun, 2021; Bindyalaxmi et al., 2022). As a continuation of maceration, MAE can lead to bixin and norbixin degradation. Therefore, maceration with distilled water as a solvent without *MAE* is already adequate for extracting bixin and norbixin from annatto seeds.

Color of annatto extract

Color is a fundamental aspect of visual experience that aids in object segmentation, food source identification, and emotional signaling (Cohen et al., 2020). It is also an extrinsic factor influencing consumer preferences when selecting products (Muniz et al., 2023). The color of the annatto extract obtained through a combination of maceration and MAE methods at various solvent pH levels and the control (maceration) is shown in Table 2. The L value represents lightness, ranging from 0 (black) to 100 (white). The a* value indicates the chromatic reflection of red-green, where red ranges from 0 to 100 and green from (0 to -80). The b* value represents the chromatic reflection of blue-yellow, where yellow ranges from (0 to 70) and blue from (0 to -70) (Wiriani et al., 2020).

The extraction of annatto using the *MAE*-assisted maceration method resulted in different L* values (lightness). The highest lightness of annatto extract was obtained with distilled water at pH 4 for 2 minutes of extraction. However, it was not significantly different from the control (extraction performed only by

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maceration), even though the *MAE*-assisted maceration method generally produced higher lightness. Acidic solutions cause vacuole walls to break more easily, allowing pigments to be released more efficiently (Pratiwi & Priyani, 2019). The higher the extracted pigment content, the more significant the increase in lightness; however, longer extraction times lead to pigment degradation. An increase in solvent pH and extraction time could result in a decrease in lightness. The reduction in lightness (darker color) may also be due to flavonoid compounds. Annatto contains flavonoid compounds (Handayani et al., 2021). Flavonoid compounds contribute to the reduction of lightness in propolis extracts (Suci & Ismawati, 2017).

Table 2. Color of annatto extract produced using *MAE-assisted maceration method*

Treatment		L	a*	b*
pH of distilled water	Extraction time (minutes)			
4	2	26.53±7.50 ^{c}	11.86±4.32^{a}	13.55±6.90^{a}
	4	25.84±5.98 ^{c}	13.46±5.81^{a}	13.18±7.07^{a}
	6	23.44±4.76 ^{bc}	14.58±7.72^{a}	11.57±8.30^{a}
7	2	24.28±5.52 ^{b}	12.50±6.55^{a}	12.13±8.50^{a}
	4	21.81± 3.75^{ab}	14.82±8.29^{a}	13.32±8.00^{a}
	6	24.29±6.56 ^{b}	17.87±8.63^{b}	12.36±8.80^{a}
9	2	24.22±8.08 ^{b}	12.75±5.31^{a}	11.24±7.81^{a}
	4	21.47± 1.77^{a}	15.40±7.92^{a}	12.85±8.97^{a}
	6	24.16±4.86 ^{b}	15.05±6.27^{a}	12.23±8.18^{a}
Control (pH 6.6)		24.64±5.98 ^{bc}	13.24±6.89^{a}	13.02±7.43^{a}

Explanation: Numbers followed by the same superscript letter in the same column indicate no significant difference at the 5% level according to the DMRT test.

The highest red color intensity was obtained using the *MAE*-assisted maceration method in a neutral solution with an extraction time of 6 minutes. Extraction with *MAE*-assisted maceration at pH 7 for 6 minutes could extract components responsible for the red color. The primary pigment components of annatto include bixin and norbixin as primary pigments, along with bixin dimethyl ester, lycopene, and several types of apocarotenoids (Chuyen & Eun, 2021). Other pigments present in annatto include β-carotene and lutein (Pillai et al., 2018). Bixin and norbixin are suspected to be more soluble in a neutral solution, and increasing the extraction time enhances the number of dissolved pigments. Therefore, the dissolution of additional pigments beyond bixin and norbixin in the neutral solution for 6 minutes contributed to the increased red color intensity of the annatto pigment.

The yellow color intensity of annatto extract obtained using maceration and *MAE*-assisted maceration methods did not show a significant difference. The components responsible for the yellow color in annatto are presumed unaffected by the applied process. The visible color of a material is composed of various color-forming components influenced by processing conditions.

Maximum absorbance

Pigments are chemical compounds capable of absorbing light within the visible wavelength range (Naselia et al., 2020). The absorbance of annatto extract measured at wavelengths of 300, 350, 400, 450, and 500 nm is presented in Table 3. The extraction of annatto was carried out using the maceration method (control), and the maceration was assisted by the *MAE* method. The solvent pH and extraction time resulted in a maximum wavelength (λ) of 300 nm, indicating the highest absorption band in the UV region. At the maximum λ (300 nm), the absorbance of the annatto extract showed a significant difference, whereas at higher wavelengths (350 nm to 500 nm), there was no significant difference. This indicates that the annatto molecules extracted using distilled water absorb light most strongly at 300 nm. The light absorption of annatto extracted through maceration assisted by *MAE* using distilled water decreases as the wavelength increases. The solvent affects the shift of the maximum λ (Sahri et al., 2019). Annatto extracted using chloroform as a solvent has a maximum λ at 501 nm, while annatto extracted using potassium hydroxide has a maximum λ at 483 nm (Franklin et al., 2023). annatto extracted using distilled water in this study has a lower λ than chloroform and potassium hydroxide as solvents.

Table 3. The absorbance of annatto extract measured at various wavelengths (λ)

Treatment		λ (nm)				
pH of distilled water	Extraction time (minutes)	300	350	400	450	500
4	2	1.42±0.54^{b}	1.03±0.44^{a}	0.898±0.50^{a}	0.766±0.49^{a}	0.649±0.44^{a}
	4	1.46±0.53^{b}	1.05±0.39^{a}	0.916±0.44^{a}	0.779±0.44^{a}	0.682±0.43^{a}
	6	1.59±0.52^{c}	1.13±0.38^{a}	0.955±0.42^{a}	0.805±0.41^{a}	0.687±0.37^{a}
7	2	1.26±0.54^{a}	0.90±0.35^{a}	0.771±0.36^{a}	0.657±0.33^{a}	0.558±0.30^{a}
	4	1.33±0.62^{a}	0.96± 0.43^{a}	0.818±0.40^{a}	0.696±0.36^{a}	0.595±0.32^{a}
	6	1.60±0.20^{c}	1.18±0.03^{a}	0.982±0.08^{a}	0.826±0.11^{a}	0.706±0.13^{a}
9	2	1.40±0.65^{a}	0.98±0.45^{a}	0.829±0.43^{a}	0.708±0.40^{a}	0.604±0.35^{a}
	4	1.49±0.67^{b}	1.06±0.43^{a}	0.913±0.40^{a}	0.772±0.34^{a}	0.660±0.31^{a}
	6	1.55±0.53^{b}	1.09±0.32^{a}	0.925±0.27^{a}	0.768±0.24^{a}	0.628±0.23^{a}
Control (pH 6.6)		1.46±0.54^{b}	1.08±0.59^{a}	0.976±0.61^{a}	0.855±0.59^{a}	0.748±0.35^{a}

Explanation: Numbers followed by the same superscript letter in the same column indicate no significant difference at the 5% level according to the DMRT test.

The principle of color measurement using the absorbance method is to determine the amount of light absorbed by a substance at a specific wavelength. Bixin, the main component of annatto, has higher solubility in basic solvents, while cell vacuole breakdown occurs more easily under acidic conditions. Increasing the extraction time at all solvent pH levels measured at λ 300 nm increases absorbance as more compounds from annatto are extracted into the solvent. Besides pigments, annatto also contains carbohydrates, proteins, and ash, with respective levels of 10.34%, 69.35%, and 3.82% (Taham et al., 2015), which can be extracted in distilled water, thereby increasing the absorbance of the extract.

Conclusion

Both maceration-assisted *MAE* extraction and simple maceration using distilled water, with or without pH adjustment, are effective for extracting annatto pigments. The highest total bixin and norbixin pigment content reached 1.56%. Higher reddish color intensity was achieved using maceration-assisted *MAE* with a neutral solution for 6 minutes. The annatto extract obtained through maceration-assisted *MAE* and simple maceration using distilled water showed maximum absorption at λ 300 nm.

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