RESEARCH ON PRODUCTION
OF SOME NATURAL FOOD COLORINGS

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Abstract. Stemming from the general trend of the world in the use of products from nature, three natural food colorings have been produced, namely the red from Momordica cochinchinensis fruits, the yellow from Gardenia jasminoides seeds, and the green from Boehmeria nivea (Ramie) leaves. Unlike industrial techniques using non-edible organic solvents, as well as traditional techniques using hot or cold water extraction, this study used ethanol 96% as a solvent for extraction. The results showed that the use of ethanol without adding water for extraction has obtained the colorings with the highest efficiency. The extraction parameters are as follows: 50 mL of ethanol, 1.0 g of dry materials, extraction temperature of 70 °C, extraction time of 60 minutes for the red and yellow colorings, and 90 minutes for the green coloring. All studied colorings were stable when heated to 90 °C for 60 minutes. The studied colorings were more stable at 4 °C in a refrigerator than at room temperature with the presence of light. They were also more stable when having been stored in ethanol than having been stored in water. After being stored for 6 days in ethanol, the weight of the red, yellow, and green colorings decreased by 2%, 0.4%, and 9.6%, respectively.

Keywords: food colorings, extraction, Momordica cochinchinensis, Gardenia jasminoides, Ramie

1 Introduction

Food colorings are additives used a lot in food processing. Color is one of the indicators of food sensory quality and contributes to an increase in appetite, stimulating appetite although food colorings are often not foods with nutritional values [1]. Food colorings include natural colorants and synthetic colorants. Recent studies have shown that artificial colorings often raise concerns for human health [2, 3]. Therefore, they have been banned or restricted for use in

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many countries, including Vietnam. Even all of the 9 currently US-approved food colorings are also thought to be related to some issues for human health. Erythrosine (Red 3) can cause cancer in animals. Benzidine or other carcinogens have been found in allura red (Red 40), tartrazine (Yellow 5), and sunset yellow (Yellow 6). Brilliant blue (Blue 1), Red 40, Yellow 5, and Yellow 6 cause hypersensitivity reactions. Yellow 5 causes genotoxicity for rodents [3, 4]. Unlike synthetic colorings, natural colorants are extracted from natural sources and harmless to human health. Natural colorings are easy to make, cheap, easy to use and do not cause strange taste for food. Using natural colorings not only improves the appearance but also improves the nutritional value of the food. Some food colorants also have pharmacological effects such as memory improvement, anticonvulsant and nerve protection [5, 6], anti-depressant [7, 8], antioxidant [9], and anti-tumor [10]. Therefore, many countries in the world have been extracting and using natural products to produce food colorings in order to replace synthetic colorings [11]. In Vietnam, most of the food colorings have been imported. Due to the requirements of food safety and hygiene, Vietnam only allows the import and use of food colorings in limited quantities [12].

Nowadays, due to the difficulties in controlling the origin of food and the general trend of the world in the use of natural products, the studies on the extraction of natural food colorings are increasingly attracting the attention of scientists. However, this is a big challenge because synthetic colorants are more stable than natural colorants when used in realistic environments such as temperature, light, pH, and oxygen [11]. As a result, the studies on the extraction of natural food colorings have great interest.

Since ancient times, humans have known the use of food colorings from nature (Table 1). The main used methods are hot or cold extraction with water (Pandanus amaryllifolius, Peristrophe roxburghiana, Curcuma longa, Ramie), or with cooking oils (Momordica cochinchinensis). In addition, some colorants are used directly from the root (Curcuma longa), or leaves (Ramie) [12]. According to modern science, natural food colorings, particularly from fruits and vegetables, are generated mainly as four groups of pigments: the green chlorophylls, the yellow-orange-red carotenoids, the red-blue-purple anthocyanins, and the red betanin [13, 14].

Table 1. Traditional natural colorants [12]

<table>
<thead>
<tr>
<th>Colors</th>
<th>Natural sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td><em>Momordica cochinchinensis</em>, Red beet, <em>Hibiscus sabdariffa</em></td>
</tr>
<tr>
<td>Purple</td>
<td><em>Peristrophe roxburghiana</em>, <em>Basella alba</em>, <em>Rhodomyrtus tomentosa</em></td>
</tr>
<tr>
<td>Yellow</td>
<td><em>Curcuma longa</em>, <em>Buddleia officinalis Maxim</em>, <em>Tagetes erecta</em></td>
</tr>
<tr>
<td>Green</td>
<td><em>Pandanus amaryllifolius</em>, <em>Sauropus androgynus</em></td>
</tr>
<tr>
<td>Black</td>
<td><em>Ramie</em>, <em>Liquidambar formosana</em></td>
</tr>
</tbody>
</table>
In this study, three basic food colorings have been produced from natural sources, namely the red from *Momordica cochinchinensis* fruits, the yellow from *Gardenia jasminoides* seeds, and the green from *Ramie* leaves (Fig. 1). These natural sources are used by humans for coloring food [12]. Traditionally in Vietnam, *Momordica cochinchinensis* fruits are used as a red coloring component in rice dishes; *Gardenia jasminoides* seeds are used as a yellow coloring component in cake dishes; and *Ramie* leaves are used as a black coloring component in cake dishes. They are not only nontoxic but also provide valuable nutrients or have pharmacological effects [15]. Carotenoids in *Momordica cochinchinensis* fruits, including lycopene and β-carotene, are highly effective antioxidants, responsible for the reduction of cancers, cardiovascular diseases, and macular dysfunction. Lycopene is the precursor to produce vitamin A, important for the development of cell membranes, immunity, and skin health [16]. *Gardenia jasminoides* seeds are used for the treatment of inflammation, jaundice, headache, edema, fever, hepatic disorders, and hypertension. In addition, crocins in *Gardenia jasminoides* seeds are used for anti-carcinogenic activity and other pharmacological studies [17]. *Ramie* leaves are used to treat miscarriages [18], T2 diabetes, cancer, and neurodegenerative diseases [19]. The *Ramie* leaves extract exhibit potential anti-HBV activity [20] and the hepatoprotective and antioxidative effects [21].

![Image of natural food colorings](image_url)

*Fig. 1.* Red coloring from *Momordica cochinchinensis* fruits, yellow coloring from *Gardenia jasminoides* seeds, and green coloring from *Ramie* leaves.
In addition, three food colorings obtained from this study have been extracted from natural sources with food grade ethanol at 70 °C. This method does not use any other chemicals like recent studies [22, 23]. The obtained products could be used directly in households without any further processing. This method provides greater extraction efficiency for these colorants, better color fastness than water extraction, and easier mixing with the foods than cooking oil extraction.

2 Materials and methods

2.1 Instruments

All absorption spectra were recorded on a Shimadzu UV-1800 UV-vis spectrophotometer.

2.2 Materials and chemicals

Ethanol 96% used for extraction is food grade ethanol. *Momordica cochininchensis* fruits, *Gardenia jasminoides* seeds, and *Ramie* leaves were purchased in Hue City, Vietnam.

For *Momordica cochininchensis* fruits, fresh fruits with red rind, evenly hatched spines, and red ripe intestine were chosen. The seeds and membrane surrounding the seeds were dried at 70 °C for 20 minutes. Then, the membrane surrounding the seeds was separated and dried at 110 °C for 60 minutes (the moisture was below 5%), cooled to room temperature, and used to extract the red coloring.

For *Gardenia jasminoides* seeds, fresh yellow fruits were chosen. The seeds were rinsed with water, dried at 110 °C for 60 minutes (the moisture was below 5%), cooled to room temperature, ground into fine flour, and used to extract the yellow coloring.

For *Ramie* leaves, fresh green leaves were chosen, rinsed with water, boiled with hot water at 100 °C for 10 minutes. Then, the leaves were dried completely at 110 °C (the moisture was below 5%), cooled to room temperature, ground into fine flour, and used to extract the green coloring.

2.3 Experimental design

**Experiments to investigate the conditions for colorings extraction**

The colorings were extracted from dry materials under the following conditions:

The volume of solvent was 50 mL. The ethanol/water volume ratios were 50/0, 40/10, 30/20, 20/30, 10/40, and 0/50; the materials weight was 0.5, 1.0, 1.5, and 2.0 (g); the extraction temperature was 30, 50, 70, and 90 °C; the extraction time was 45, 60, 75, 90, 120, and 150 minutes.
Experiments to investigate the durability of the colorings

The colorings were prepared in water and ethanol.

The samples of colorings were stored in two different conditions: at room temperature with the presence of light and at 4 °C in a refrigerator.

The durability of the colorings was evaluated through the reduction of its weight after being stored for 2, 4, and 6 days.

2.4 Method for quantification of colorings

Method for quantification of red coloring

The red coloring from *Momordica cochinchinensis* fruits was quantified on the basis of the content of carotenoid, as follows [24]:

\[
\begin{align*}
    m_{\text{carotenoid}} &= C_{\text{carotenoid}} \cdot V \cdot F \\
    C_{\text{carotenoid}} &= (1000 \cdot A_{470} - 1.43 \cdot C_a - 35.87 \cdot C_b)/205 \\
    C_a &= 13.36 \cdot A_{664} - 5.19 \cdot A_{649} \\
    C_b &= 27.43 \cdot A_{649} - 8.12 \cdot A_{664}
\end{align*}
\]

where \( m_{\text{carotenoid}} \) is the weight of carotenoid (g); \( C_{\text{carotenoid}} \) is the concentration of carotenoid (g/L, in ethanol); \( V \) is the volume of the extracted solution (L); \( F \) is the dilution ratio from the extracted solution to measure absorbance; \( A_{470}, A_{664}, A_{649} \) are the values of the absorbance at wavelengths of 470, 664, and 649 nm.

Method for quantification of yellow coloring

The yellow coloring from *Gardenia jasminoides* seeds was quantified on the basis of the content of crocin [25, 26], as follows [27, 28]

\[
m_{\text{crocin}} = A_{440} \cdot M \cdot V \cdot F/(\varepsilon \cdot l)
\]

where \( m_{\text{crocin}} \) is the weight of crocin (g); \( A_{440} \) is the absorbance at a wavelength of 440 nm; \( M \) is the molecular weight of crocin (977 g/mol); \( V \) is the volume of the extracted solution (L); \( F \) is the dilution ratio from the extracted solution to measure absorbance; \( \varepsilon \) is the molar extinction coefficient of crocin in ethanol (89000 L · cm⁻¹ · mol⁻¹); \( l \) is the base thickness of absorption cell (\( l = 1 \text{ cm} \)) [29].

Method for quantification of green coloring

The green coloring from *Ramie* leaves was quantified on the basis of the content of chlorophyll a (Chla), chlorophyll b (Chlb), and chlorophyll (a+b) (Chla+b), as follows [30]

\[
C_a = 16.29 \cdot A_{665} - 8.54 \cdot A_{652}
\]
\[ C_b = 30.66 \cdot A_{652} - 13.58 \cdot A_{665} \]  
(7)

\[ Ca+b = 22.12 \cdot A_{652} + 2.71 \cdot A_{665} \]  
(8)

where \( C_a, C_b, C_{a+b} \) are the concentration of chlorophyll a, chlorophyll b, and chlorophyll (a+b) (g/L); \( A_{665}, A_{652} \) are the values of the absorbance at wavelengths of 665 and 652 nm.

2.5 Statistical processing method

The obtained experimental results are presented as mean values and standard deviations. The differences between samples due to the change of the studied factors were assessed by using the one-way analysis of variance (ANOVA) calculations for the comparison of the mean values [31].

3 Results and discussion

3.1 Conditions of production of natural food colorings

Effect of solvents on the efficiency of colorings extraction

The extraction of colorings was investigated with various ethanol/water volume ratios as shown in Table 2.

For the red coloring extraction from *Momordica cochinchinensis* fruits, the one-way ANOVA calculations show that the calculated value of \( F \) is 409, the critical value of \( F \) (for a one-tailed test) is 3.106 \((p = 0.05, f_1 = 5, f_2 = 12)\), and the least significant difference is 0.126 (the critical value of \( t \) is 2.18, \( p = 0.05, f = 12 \)). Since the calculated value of \( F \) is greater than the critical value, the ethanol/water volume ratio is believed to affect the weight means of carotenoids. When the ethanol/water volume ratios change from 50/0 to 20/30, the difference between mean values is greater than the least significant difference (0.126), so the weight means of carotenoids are considered as having a significant difference. Then, the ethanol/water volume ratios further decrease from 20/30 to 0/50, the difference between mean values is less than the least significant difference, so the weight means of carotenoids are considered as having a significant difference. As a result, the efficiency of red coloring extraction is greatest when the solvent used for extraction is pure ethanol.

For the yellow coloring extraction from *Gardenia jasminoides* seeds, the one-way ANOVA calculations show that the addition of water to extract the coloring has a significant effect and reduces the efficiency of the extraction process. This is supported by the fact that the calculated value of \( F \) is 5334.96, greater than the critical value of \( F \) (3.106, \( p = 0.05, f_1 = 5, f_2 = 12 \)), and all the differences between the mean values are greater than the least significant difference of 0.118. These results show that the use of pure ethanol for extraction provides the yellow coloring with the highest yield.
Table 2. Effect of solvents on the efficiency of colorings extraction

<table>
<thead>
<tr>
<th>N°</th>
<th>Ethanol (mL)</th>
<th>Water (mL)</th>
<th>Carotenoids (mg)</th>
<th>Crocin (mg)</th>
<th>Chlorophyll (a+b) (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Means (SDn=3)</td>
<td>Means (SDn=3)</td>
<td>Means (SDn=3)</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>0</td>
<td>2.303 (0.102)</td>
<td>13.738 (0.076)</td>
<td>13.845 (0.054)</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>10</td>
<td>1.627 (0.085)</td>
<td>9.343 (0.081)</td>
<td>3.781 (0.099)</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>20</td>
<td>0.978 (0.112)</td>
<td>7.857 (0.064)</td>
<td>3.676 (0.112)</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>30</td>
<td>0.442 (0.007)</td>
<td>7.455 (0.075)</td>
<td>3.540 (0.040)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>40</td>
<td>0.344 (0.006)</td>
<td>6.821 (0.035)</td>
<td>3.437 (0.018)</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>50</td>
<td>0.220 (0.010)</td>
<td>5.937 (0.055)</td>
<td>3.264 (0.074)</td>
</tr>
</tbody>
</table>

* Materials: 1 g, extraction temperature: 70 °C, extraction time: 60 min for *Momordica cochinchinensis* fruits and *Gardenia jasminoides* seeds, 90 min for *Ramie* leaves.

For the green coloring extraction from *Ramie* leaves, the one-way ANOVA calculations give the calculated F value of 9746.93, and the least significant difference is 0.132. In this case, the critical value of F is 3.106 (p = 0.05, f₁ = 5, f₂ = 12), less than the calculated value of F, therefore, the change in the solvents ratio is thought to have a significant impact on the extraction process. The extraction efficiency is greatest when using only ethanol for extraction. The addition with any volume of water also significantly reduces the extraction efficiency. When the ethanol/water volume ratios change from 40/10 to 0/50, the difference between the mean values is less than the least significant difference, so the weight means of chlorophyll (a+b) are considered as having no significant difference. This may be due to the fact that chlorophyll denatures under the effect of water and heat. As a result, using only ethanol is the best choice for green coloring extraction from *Ramie* leaves.

Effect of the material weight/solvent volume ratio on the efficiency of colorings extraction

The effect of the material weight/solvent volume ratio on the efficiency of colorings extraction was also investigated.

For the red coloring extraction, the ANOVA calculations show that the calculated value of F is 91.1, greater than the critical value of 4.066 (p = 0.05, f₁ = 3, f₂ = 8). This leads to the fact that the material weight/solvent volume ratio is believed to affect the extraction efficiency for the red coloring. The least significant difference obtained from the ANOVA calculations is 0.016 (the critical value of t is 2.31, p = 0.05, f = 8). This indicates that the significant difference of mean values only occurs between sample N°2 and sample N°3 where the difference between mean values is greater than 0.016 (Table 3). As a result, 1 g of material/50 mL of solvent ratio was chosen for the red coloring extraction.
Table 3. Effect of the material weight/solvent volume ratio on the efficiency of colorings extraction

<table>
<thead>
<tr>
<th>N°</th>
<th>Material weight (g)</th>
<th>Weight of carotenoids/weight of materials (%)</th>
<th>Weight of crocin/weight of materials (%)</th>
<th>Weight of Chlorophyll(a+b)/weight of materials (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Means (SD (n=3))</td>
<td>Means (SD (n=3))</td>
<td>Means (SD (n=3))</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.207 (0.011)</td>
<td>1.492 (0.032)</td>
<td>1.385 (0.008)</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>0.220 (0.010)</td>
<td>1.433 (0.019)</td>
<td>1.374 (0.021)</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>0.140 (0.005)</td>
<td>1.214 (0.024)</td>
<td>1.343 (0.009)</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>0.130 (0.005)</td>
<td>1.155 (0.058)</td>
<td>1.304 (0.010)</td>
</tr>
</tbody>
</table>

* 50 mL of ethanol, extraction temperature: 70 °C, extraction time: 60 min for *Momordica cochinchinensis* fruits and *Gardenia jasminoides* seeds, 90 min for Ramie leaves.

For the yellow coloring extraction, the experimental results and the one-way ANOVA calculations show that the material weight/solvent volume ratio has a significant influence on the obtained yellow coloring yield because the calculated value of *F* is 61.54, greater than the critical value of 4.066 \((p = 0.05, f_1 = 3, f_2 = 8)\). Since the difference between the mean values is less than the least significant difference of 0.068, the use of 50 mL of ethanol for 0.5 g or 1.0 g of materials provides no significant difference in the yield of yellow coloring and is better than for 1.5 g or 2.0 g of materials (Table 3). As a result, the use of 50 mL of ethanol for 1.0 g of materials was chosen for the yellow coloring extraction.

For the green coloring extraction, the obtained results from the one-way ANOVA calculations show that the calculated value of *F* is 23.71 and the least significant difference is 0.024. The calculated value of *F* is greater than the critical value of 4.066 \((p = 0.05, f_1 = 3, f_2 = 8)\). This result indicates that the material weight/solvent volume ratio has a significant influence on the efficiency of green coloring extraction. Since the difference between the mean values of sample N°1 and sample N°2 is less than the least significant difference of 0.024, and the difference between the mean values of sample N°2 and sample N°3 is greater than the least significant difference of 0.027, the use of 50 mL of ethanol for 1.0 g of materials provides an insignificant difference from the use of 50 mL of ethanol for 0.5 g of materials in the extraction efficiency. In contrast, the use of 50 mL of ethanol for 1.0 g of materials provides a significant difference in the extraction efficiency and is better than from the use of 50 mL of ethanol for 1.5 g of materials (Table 3). As a result, the use of 50 mL of ethanol for 1.0 g of materials was chosen for the green coloring extraction.

**Effect of extraction temperature on the efficiency of colorings extraction**

The obtained results from investigations of the effect of the extraction temperature on the efficiency of colorings extraction are listed in Table 4.
Table 4. The effect of the extraction temperature on the efficiency of colorings extraction

<table>
<thead>
<tr>
<th>N°</th>
<th>Extraction temp (°C)</th>
<th>Carotenoids (mg)</th>
<th>Crocin (mg)</th>
<th>Chlorophyll(a+b) (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Means</td>
<td>SD (n = 3)</td>
<td>Means</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>0.513</td>
<td>0.024</td>
<td>13.410</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>1.514</td>
<td>0.038</td>
<td>13.786</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>2.184</td>
<td>0.092</td>
<td>13.868</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>2.216</td>
<td>0.016</td>
<td>13.744</td>
</tr>
</tbody>
</table>

* Materials: 1 g, 50 mL of ethanol, extraction time: 60 min for *Momordica cochinchinensis* fruits and *Gardenia jasminoides* seeds, 90 min for *Ramie* leaves.

For the red coloring extraction, the calculated value of $F$ is 707.8, greater than the critical value of 4.066 ($p = 0.05, f_1 = 3, f_2 = 8$). This shows that the extraction temperature strongly affects the efficiency of red coloring extraction. The difference of mean values between the extraction temperature of 70 °C and 90 °C is less than the least significant difference of 0.098. This result indicates that there is no significant difference in efficiency between the extraction temperature of 70 °C and 90 °C. As a result, the extraction temperature of 70 °C was chosen for the red coloring extraction.

For the yellow coloring extraction, the calculated value of $F$ is 27.15, greater than the critical value of 4.066 ($p = 0.05, f_1 = 3, f_2 = 8$). The least significant difference is 0.126. So, the extraction temperature has a significant effect on the efficiency of yellow coloring extraction. When the extraction temperature changes from 30 °C to 50 °C, the extraction efficiency increases, and then, if the temperature rises further, the extraction efficiency becomes insignificantly different. In this case, the temperature of 70 °C was selected for the yellow coloring extraction.

For the green coloring extraction, the obtained results show that the extraction temperature has a significant effect on the efficiency of green coloring extraction. This is because the calculated value of $F$ is 15672, greater than the critical value of 4.066 ($p = 0.05, f_1 = 3, f_2 = 8$). The difference between the mean values of sample N°2 and sample N°3 is greater than the least significant difference of 0.090, so there is a significant difference in efficiency between the extraction temperature of 50 °C and 70 °C. In contrast, the difference between the mean values of sample N°3 and sample N°4 is less than the least significant difference of 0.090, so there is no significant difference in efficiency between the extraction temperature of 70 °C and 90 °C. As a result, the extraction temperature of 70 °C was chosen for the green coloring extraction.

**The effect of the extraction time on the efficiency of colorings extraction**

The impact of extraction time on the efficiency of colorings extraction was also investigated, and the results are listed in Table 5. For the red coloring extraction, the calculated value of $F$ is 18.60,
greater than the critical value of 4.066 \((p = 0.05, f_1 = 3, f_2 = 8)\). So, the extraction time is said to affect the efficiency of red coloring extraction. The least significant difference obtained from the ANOVA calculations is 0.175. This suggests that there is no significant difference in efficiency between the extraction times from 60 to 90 minutes, and the extraction time of 60 minutes was chosen for the red coloring extraction.

Table 5. The effect of the extraction time on the efficiency of colorings extraction

<table>
<thead>
<tr>
<th>N°</th>
<th>Extraction time (min)</th>
<th>Carotenoids (mg)</th>
<th>Crocin (mg)</th>
<th>Chlorophyll(a+b) (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Means (SD/n = 3)</td>
<td>Means (SD/n = 3)</td>
<td>Means (SD/n = 3)</td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>1.728 0.086</td>
<td>13.377 0.119</td>
<td>5.351 0.097</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>2.176 0.070</td>
<td>13.800 0.089</td>
<td>6.118 0.113</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>2.244 0.100</td>
<td>13.779 0.151</td>
<td>8.093 0.146</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>2.118 0.111</td>
<td>13.758 0.069</td>
<td>13.865 0.111</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td></td>
<td></td>
<td>10.236 0.115</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td></td>
<td></td>
<td>9.691 0.037</td>
</tr>
</tbody>
</table>

* Materials: 1 g, 50 mL ethanol, extraction temperature: 70 °C.

For the yellow coloring extraction, the calculated value of \(F\) is 9.79, a little greater than the critical value of 4.066 \((p = 0.05, f_1 = 3, f_2 = 8)\). The least significant difference is 0.211. This suggests that when the extraction time is equal to or greater than 60 minutes, the extraction efficiency is not significantly different. Meanwhile, there is a significant difference in the extraction efficiency between the extraction time of 45 minutes and 60 minutes because in this case, the difference of the mean values is greater than the least significant difference of 0.211. Finally, the time of 60 minutes was selected for the yellow coloring extraction.

For the green coloring extraction, the ANOVA calculations give the calculated value \(F\) of 2452, greater than the critical value of 3.106 \((p = 0.05, f_1 = 5, f_2 = 12)\). The least significant difference is 0.193, less than all the differences of the mean values. This suggests that the extraction time has a significant effect on the efficiency of green coloring extraction, and there is a significant difference in efficiency between the studied extraction times. When the extraction time increases from 45 to 90 minutes, the efficiency of the green coloring extraction increases, and then, when the extraction time increases further from 90 to 150 minutes, the efficiency of the green coloring extraction decreases. Finally, the time of 90 minutes was selected for the green coloring extraction.

### 3.2 Effects of storage temperature and time on colorfastness

The results of the study on temperature and extraction time show that all studied colorings were stable at high temperatures. The colorings can be heated to 90 °C for 60 minutes without
any significant changes. Here, the effects of storage temperature and time on the colorfastness were further investigated (Figure 2).

In the case of the *Momordica cochinchinensis* fruits, the coloring was quite stable at 4 °C in a refrigerator both in ethanol and water. After being stored for 6 days, the weight of the red coloring decreased only by 2% and 3% in ethanol and water, respectively. Meanwhile, if the colorings were stored at room temperature with the presence of light, their weight decreased by 15% and 20%, respectively.

In the case of the *Gardenia jasminoides* seed, after being stored for 6 days in a refrigerator at 4 °C, the weight of the yellow coloring decreased only by 0.4% and 5% in ethanol and water, respectively. Meanwhile, these values were by 3% and 14%, respectively, if being stored at room temperature with the presence of light.

The green coloring from *Ramie* leaves was less stable than the red and yellow colorings. The weight of the green coloring decreased by 9.6% and 50% in ethanol and water, respectively, after being stored for 6 days in a refrigerator at 4 °C. The weight of the green coloring decreased

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**Fig. 2.** The effects of storage temperature and time on colorfastness.
dramatically, up to 84% and 85%, in ethanol and water, respectively, after being stored for 6 days at room temperature with the presence of light.

The results show that the colorings stored in a refrigerator at 4 °C were more stable than at room temperature with the presence of light. They were also more stable when stored in ethanol than stored in water. The red and yellow colorings (in both ethanol and water) changed insigificantly after being stored for 6 days in a refrigerator at 4°C. Meanwhile, the weight of the green coloring decreased quite significantly even when stored in a refrigerator at 4 °C and in water, and decreased sharply when stored at room temperature with the presence of light (in both ethanol and water). This may be due to the fact that chlorophyll is a powerful photochemical compound, and it is both acidic and basic and is easily oxidized and decomposed under the action of light and temperature, and therefore less stable in water than in ethanol [32].

4 Conclusions

Three food colorings – the red from Momordica cochinensis fruits, the yellow from Gardenia jasminoides seeds, and the green from Ramie leaves – were extracted from natural sources using food grade ethanol without using any other chemicals. They were not only nontoxic but also provided valuable nutrients or had pharmacological effects and could be used directly in households without any further processing. In addition, the products obtained from this study also have many other outstanding features such as high extraction efficiency, good colorfastness, and ease of use.

References


