The Food Color Extraction from Gardenia Fruit
(*Gardenia jasminodes Ellsiforma* var *gardiflora* Makino) :
The Appropriate Extraction Conditions

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**ABSTRACT**

Moisture and fat contents of the fresh and the dried gardenia fruits were investigated. The results showed that the moisture content in the fresh fruits was higher than that in the dried fruits and the fat content in the fruits was higher than that in the pericarp. It was also found that the fat content did affect the color extraction efficiency. Methanol and water were the best solvents for color extraction. Addition of acid to the solvents did increase the solubility of the color pigments but decreased the stability of the color extract.

The appropriate temperature and time needed for extraction when water employed as the extraction solvent were 70-80°C and 5-10 minutes respectively. The extraction with stirring process did not increase the extraction efficiency. However, when methanol was used as the extraction solvent, the efficiency of extraction increase and the appropriate temperature and time for extraction was 60°C and 30 minutes respectively. The appropriate volume of solvent, either water or methanol for 1 g Gardenia fruit sample was 200 milliliters.

According to the results carried out by TLC, UV-Spectrophotometer, Infra-Red Spectrophotometer and Nuclear Magnetic Resonance, they revealed that the gardenia fruit color extract was Crocin. The evaluation of gardenia fruit color extract as food colorant indicated that the color extract could be used as food colorant i.e. yellow food color.

**Key words** : fruits color, gardenia color, appropriate extraction

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**INTRODUCTION**

Color is the first sensory quality by which food are judged, since food quality and flavor are closely associated with color. In addition, many colorless convenience foods such as confectionary products, snacks, beveragas and gelatin desserts, the food color was added in order to identify the taste and increased the palatability.

The color generally added to the food is either natural or synthetic food colors. Since more and more synthetic food colors were reported unsafe (FAO, 1963 ; FAO,1985 ; JECFA,1964), therefore, the search for more natural food colors is of vital importance. The objective of this study was to find out whether the yellow pigment in Gardenia fruit could be used as the food colorant and to replace tartrazine or FD and C yellow No.5 whose safety for human consumption is presently found controversy (Weissler, 1975 ; Swain *et al.*, 1984).

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In addition, the increasing used of the native gardenia fruit can not only generate more income to the local growers but also help to reduce the foreign currency which had to pay for food color imported.

**MATERIALS AND METHODS**

Fresh gardenia fruit were purchased from Nakorn-Nayok province and dried gardenia fruit, prepared by Blanching the fresh gardenia fruit before sun drying.

1. **Moisture and fat contents**

Moisture and fat contents of the fresh whole gardenia fruit, the dried whole gardenia fruit fruits, the fresh gardenia fruit (without pericarp) and the dried gardenia fruit without pericarp were carried out according to AOAC (1984).

2. **Comparison of the Gardenia fruit color**

Extracted from both the fresh and the dried gardenia fruit were performed by soaking different 0.1 g of the above mentioned samples in to 35 ml distilled water for 5, 10, 20, 30 and 60 minutes. The filtrates were drawn for absorbance at 440 nm Measurement.

3. **The effects of different fat contents on**

The color extraction efficiency of the dried gardenia fruit were studied by soaking the dried fruits in petroleum ether for 1 hour, prior to filtering. The same procedure was repeated 3 times. Five different portions of samples obtained would contain different quantities of fat. Each of these samples was divided into 2 parts, the first part for fat determination according to AOAC (1984) and the second part for food color extraction by soaking 0.1 g of sample in 35 ml of distilled water for 10 minutes.

4. **The investigation on appropriate solvent, time, temperature and volume of solvent used**

For food color extraction were conducted. The gardenia fruit used in this experiment was dried ground and defatted.

4.1 **Solvents**

Different solvents, i.e. distilled water; 10, 20 and 30% acetic acid; 0.1, 1.0, 3.0 and 5.0% hydrochloric acid; methanol; 0.1 and 1.0% hydrochloric acid in methanol; ethanol; 0.1 and 1.0% hydrochloric acid in ethanol were used for color extraction. The identical soaking time was 10 minutes. The intensity of the food color extract was measured by UV-Spectrophotometer at 440 nm.

4.2 **Time and Temperature**

The studies were carried out by soaking different portions of 0.1 g ground gardenia fruit in 35 ml distilled water and incubated in the waterbath at 40, 50, 60, 70, 80 and 100 °C and room temperature (32 °C). The samples were drawn for measuring the absorbance at 440 nm by UV-Spectrophotometer after 0, 1, 2, 5, 10, 20, 30 and 60 minutes extraction. The same procedures were repeated by using methanol as the extraction solvent.

4.3 **Volume of solvent**

The solvents selected for this investigation were water and methano. Different portions of 0.1 g ground gardenia fruit were extracted for 10 min. in various volumes of water and methanol, i.e. 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 milliliters. The food color extracts were drawn for absorbance measurement at 440 nm by UV-Spectrophotometer. The extraction procedures were carried out by soaking in the solvent only and together with stirring process.

5. **Separation and identification of color**

Components of the gardenia fruit color extract were carried out by using Thin Layer Chromatography and Spectroscopy, respectively.

5.1 **Separation of Gardenia fruits color**

Extract by Thin Layer Chromatography

Sixteen different gardenia fruit color extracts, based on raw materials and kind of solvent (water or methanol) used, i.e. fresh whole gardenia fruit, dried whole gardenia fruit, fresh gardenia fruit without pericarp, dried gardenia fruit without pericarp, defatted fresh gardenia fruit, defatted dried gardenia fruit, defatted fresh gardenia fruit
without pericarp and defatted dried gardenia fruit without pericarp were separated by Thin Layer Chromatography. Silica gel 60 g was used as a stationary phase. The appropriate solvent systems studied were ethyl acetate : 2-propanol : water (10:6:3) (Pfander and Wittwer, 1975), chloroform : methyl alcohol (8:1), acetone : 1-pentanol : water (1:2:10), butyl alcohol : acetic acid : water (4:1:1), chloroform : acetic acid (9:1) (Duguenois, 1972), benzene : methyl alcohol (95:5) (Corradi et al., 1981), benzene : ethyl acetate (3:7), 2.5% sodium chloride solution (Pavanch, 1972), ethyl acetate : 1-pentanol : water (1:2:1), methyl alcohol : acetone (5:2), 1-pentanol : acetic acid : water (4:1:1), 1-pentanol : acetic acid : water (5:1:1). The ascending chromatography was employed.

5.2 Identification of the color extract components.

The dried color extract components separated were scrapped from the Thin Layer plate. The appropriate volume of ethanol was added and the silica gel was separated by IEC Clinical Centrifuge. Solution of the color extract was divided into 2 portions, one for measuring by UV-Spectrophotometer, another was examined by Infra-Red Spectrophotometer and Nuclear Magnetic Resonance after evaporated under vacuum.

6. The evaluation of the gardenia fruit color extract as food colorant was carried out by dissolving the Gardenia fruit color extract powder in propylene glycol and glycerol. Both color extract solutions were then used as coloring agents for synthetic pineapple flavored beverage and Kha-nom Sali (Thai traditional dessert). The different of color, flavor and texture of the tasted food were observed compared to the ones using Tartrazine (Amerine et al., 1965).

RESULTS AND DISCUSSION

The results of moisture and fat contents of the Gardenia fruit showed that the moisture content in the fresh Gardenia fruit was higher than that in the dried Gardenia fruit and the fat content in the fruits was higher than that in the pericarp, i.e. the average fat content in the Gardenia fruit without pericarp was 32.0% and 21.1% for the whole fruits. The moisture and fat contents of the Gardenia fruit examined were tabulated in Table 1.

Results of the color extraction from the fresh and the dried Gardenia fruit indicated that the extract from the fresh gave the higher color intensity (Figure 1). The data also showed that the longer the extraction time, the more the concentration of the gardenia fruit color extracted from either the dried or the fresh gardenia fruits. Owing to the high fat content of the gardenia fruit studied, the efficiency of the color extraction might be interfered. Therefore, the investigations on the color extraction from the gardenia fruit with different levels of fat content were performed. The data obtained indicated that there was a relationship between the fat content

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture(%)</th>
<th>Fat content(%)</th>
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</thead>
<tbody>
<tr>
<td>Dried gardenia fruit without pericarp</td>
<td>6.5</td>
<td>30.4</td>
</tr>
<tr>
<td>Dried whole gardenia fruit</td>
<td>6.0</td>
<td>23.9</td>
</tr>
<tr>
<td>Fresh gardenia fruit without pericarp</td>
<td>51.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Fresh whole gardenia fruit</td>
<td>56.7</td>
<td>21.1</td>
</tr>
</tbody>
</table>

Table 1 Moisture and fat contents of the gardenia fruit.
in the fruits and the concentration of the color extracted, i.e. the higher the fat content in the extracted sample, the lower the concentration of the color extracted (Figure 2).

The observations on appropriate color extraction solvents are shown in Figure 3. Methanol gave the highest extraction efficiency over water and ethanol respectively confirmed by the study of Yu et al. (1975) and Nobuo et al. (1976). Adding acetic acid or hydrochloric acid to the solvent did improve the efficiency of the extraction. However, the degradation of color extract occurred, if the concentration of the acid was too high. Data obtained from the studies on the optimum time and temperature for gardenia fruit color extraction by water (Figure 4) showed that the efficiency of the color extraction increased with the increasing extraction temperatures, but only in a very short period of time. However, prolonged extraction at higher temperature resulted in the marked reduction of the color extraction efficiency. The optimum

![Figure 2](image2.png) Relationship between the color extraction efficiency and the fat content of the dried gardenia fruit.

![Figure 3](image3.png) The extractions of the gardenia fruit by various solvents.

**Figure 1** Color extraction from fresh and dried gardenia fruit. (0.1 g of sample in 35 ml distilled water).

W = water  
M = methyl alcohol  
AC = conc. acetic acid  
E = ethyl alcohol  
HC = conc. hydrochloric
temperature and time for extraction were 70-80°C and 5-10 minutes respectively. The extraction with stirring process did not increase the extraction efficiency. However, when methanol was used as the extraction solvent, the efficiency of extraction increased either with or without stirring process.

Results from the investigations on the optimum volume of solvents (water and methanol) for the gardenia fruit color extraction (Figure 5) indicated that the extraction efficiency increased with the increasing volumes of the solvent. Soaking of the samples in water with and without stirring resulted in no difference in the color extraction efficiency. But a marked difference showed when methanol was used as the solvent. The optimum volume of solvent, either water or methanol for 1 g gardenia fruit sample was 200 milliliters.

The studies on the solvent system for the separation of 16 different color extracts by Thin Layer Chromatography (Figure 6) indicated that the appropriate solvent system was 1-pentanol : acetic acid : water in the ratio of 4:1:1 and developing time was 6 hours. The results also showed that each of the 16 different color extracts were separated into 6 points and all were yellow. The point No.4 had the highest intensity.

Identification of color extract component carried out by UV-Spectrophotometer showed that all 6 points had similar absorbance which indicated that they should contain the same compounds or derivatives (Table 2).

According to results obtained from the absorbance measurement by UV-Spectrophotometer indicated that all the 6 points of the gardenia fruit extract separated had the same molecular structure. Therefore, the point No.4 which had the highest intensity was selected for further examination by the Infra-Red Spectrophotometer. The results in Figure 7 showed that ir spectrum (in KBr) : 945 cm⁻¹ (-CH=CH-trans), 1050 cm⁻¹

**Figure 4** Appropriate time and temperature for the gardenia fruit color extraction by water (0.1 g of sample in 35 ml of distilled water).

**Figure 5** Appropriate solvent volume and extraction procedure.
(-C-O-CO, ether), 1160 cm\(^{-1}\) (-C-O-, ester), 1562
and 1618 cm\(^{-1}\) (-C=C-, conjugated polyene), 2920
cm\(^{-1}\) (CH-aliphatic) and 3420 cm\(^{-1}\) (-OH). The
color samples investigated by NMR were prepared
dissolving the point No. 4 color with DMSO and
CHCl\(_3\), nmr spectrum shown in Figure 8 were 8.06
(CHCl\(_3\)), 7.66d[2H, H at C(10)] and C(10)7.2-
7.5d(8H of polyene), 5.43(2H, H at C(1) of glucose),
5.2-5.35d[6H, H at C(2)-C(4) of glucose), 4.9-
5.1d(H of OH of glucose), 4.17d(H at C(7) of
gentiobiose), 2.56d(DMSO), 1.86d(12H, H of me-
thyl), 1.26d(H\(_2\)O).
The absorbance, ir and nmr spectrums
studied were found comparable to the absorbance,
ir and nmr spectrums of Crocetin investigated by
Pfander and Wittwer (1975). In addition, the Rf of

Figure 6 Separation of the gradenia fruit color extracts by TLC.
A = Fresh gardenia fruit (defatted) (extracted by methanol)
B = Fresh gardenia fruit (defatted) (extracted by water)
C = Fresh gardenia fruit without pericarp (defatted) (extracted by methanol)
D = Fresh gardenia fruit without pericarp (defatted) (extracted by water)
E = Fresh whole gardenia fruit (extracted by methanol)
F = Fresh whole gardenia fruit (extracted by water)
G = Fresh gardenia fruit without pericarp (extracted by methanol)
H = Fresh gardenia fruit without pericarp (defatted) (extracted by water)
I = Dried gardenia fruit (defatted) (extracted by methanol)
J = Dried gardenia fruit (defatted) (extracted by water)
K = Dried gardenia fruit without pericarp (defatted) (extracted by methanol)
L = Dried gardenia fruit without pericarp (defatted) (extracted by water)
M = Dried whole gardenia fruit (extracted by methanol)
N = Dried whole gardenia fruit (extracted by water)
O = Dried gardenia fruit without pericarp (extracted by methanol)
P = Dried gardenia fruit without pericarp (extracted by water)
Table 2  Absorbance of the gardenia fruit color extract components scrapped from thin-layer chromatography.

<table>
<thead>
<tr>
<th>No. of color point</th>
<th>Maximum wavelength for maximum absorbance (λ\text{Max MeOH, in methanol})</th>
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<tbody>
<tr>
<td>No.1</td>
<td>423.4, 445.7 and 470.9 nm</td>
</tr>
<tr>
<td>No.2</td>
<td>422.8, 445.9 and 471.2 nm</td>
</tr>
<tr>
<td>No.3</td>
<td>423.2, 445.3 and 471.0 nm</td>
</tr>
<tr>
<td>No.4</td>
<td>423.3, 445.6 and 471.0 nm</td>
</tr>
<tr>
<td>No.5</td>
<td>423.2, 445.3 and 471.0 nm</td>
</tr>
<tr>
<td>No.6</td>
<td>422.5, 445.1 and 471.0 nm</td>
</tr>
</tbody>
</table>

Figure 7  Ir-spectrum of color pigment separated from the gardenia fruit color extract (KBr).

Figure 8  Nmr-spectrum of color pigment separated from the gardenia fruit color extract (in CHCl₃ and DMSO).
the gardenia fruit color extract studied by TLC, the color point No.4 was 0.44 which was comparable to \( R_f = 0.5 \) of crocetin-(b-D-gentiobiosyl)-(b-D-glucosyl)-ester (Pfander and Wittwer, 1975). Therefore, the color point No.4 could be crocetin-(b-D-gentiobiosyl)-(b-D-glucosyl)-ester.

According to the results obtained from the investigation carried out by TLC, UV-Spektrophotometer, Infra-Red Spectrophotometer and NMR, it could be concluded that the Gardenia fruit color extract was crocin (Gisvold and Rogers, 1938; Peach and Tracy, 1955; Deven and Scott, 1972; Bentley, 1960). The evaluation of Gardenia fruit color extract as food colorant was also carried out. The color extract powder was prepared in propylene glycol and glycerol. Both color samples and Tartrazine were added to various portions of the synthetic pineapple flavored beverage and Khao nom Sali. Results from organoleptic tests showed that there was no different acceptance on the color and texture of the tested foods. While there was a bitter aftertaste found in the food samples colored by the color extract in propylene glycol. Therefore, it could be concluded that gardenia fruit color extract in glycerol could be used as food colorant without any adverse effect to the food product.

**CONCLUSION**

According to the data obtained it indicated that the fat content was higher in the fruit. The best solvents for extraction were water and methanol. Addition of acid to the solvent increased the extraction efficiency but reduced the stability of the color extract. The optimum temperature and time for color extraction were 70-80°C and 5-10 minutes, when water were used as the extraction solvent. The extraction with or without stirring process did not affect the efficiency. However, when methanol was used as the extraction solvent, the extraction efficiency increased. The appropriate volume of solvent either water or methanol was 200 milliliters for 1 g gardenia fruit sample.

Based on the results carried out by TLC, UV-Spektrophotometer, Infra-Red Spectrophotometer and NMR, it could be concluded that the gardenia fruit color extract was Crocin.

The evaluation of gardenia fruit color extract as potential food colorant indicated that the color extract could be used as food colorant.

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**LITERATURE CITED**


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