Comparison of hot water and acetone extraction methods on anthocyanin content and color characteristics of butterfly pea flower extracts

Danielle M. Voss, Sydney M. Grouse, M. Mónica Giusti

ABSTRACT
The food industry is challenged by sourcing viable blue, natural pigments as there are limited sources in nature. One promising source is Butterfly Pea Flower (Clitoria ternatea (BPF)—an anthocyanin-rich natural flower historically used for food color and tea. The purpose of this study was to compare the efficiency of dried BPF pigment extraction with water to that with organic solvents (acetone). The total anthocyanin content and color characteristics of the extracts from the two procedures were compared. The results showed that hot water was an effective method for pigment extraction from dried BPF, yielding more pigment than the organic solvent under the conditions of this study. The extracts also differed by color and pH. Future research should look to optimize hot water extraction for BPF pigments.

INTRODUCTION
Food manufacturers are under increasing consumer pressure to replace synthetic colorants in food with natural counterparts. However, there are limited sources of naturally occurring blue pigments. A promising source is the butterfly pea flower (BPF), an edible blue flower originating from Asia containing unique anthocyanins with B-ring acylation patterns[1,2]. Anthocyanin extraction from plant material is efficiently done with organic solvent; however, extra processing steps are required to ensure safety for consumption. Therefore, the development of extraction procedures using safe, environmentally friendly solvents is important for the food industry.

AIM
The purpose of this experiment was to compare the efficiency of dried BPF pigment extraction with water to that with acetone. Efficiency was measured by color characteristics and total anthocyanin content. It was hypothesized that acetone would be more effective than hot water.

MATERIALS & METHODS

**Water Extraction Procedure**
- Mixed BPF with water (90°C)
- Stirred (5 min.)
- Filtered through coffee filter.

**Acetone Extraction Procedure**
- Frozen BPF
- Blended with acetone
- Filtered
- Partitioned with chloroform
- Reconstituted
- Evaporated
- Collected extract

**Color Measurements:**
CIE L\*a\*b\* was monitored by UltraScan Vis spectrophotometer under total transmittance, illuminant D65, and 10° observer angle. Full spectrum Absorbance was measured in a 2mm cuvette.

**Total Anthocyanin Content:**
A modification of the pH differential method was used; results were expressed as delphinidin-3-glucoside equivalents using MW = 465.2 g/mol and molar absorptivity = 13,000 L/mol*cm [3,4].

**RESULTS**

**Color Characteristics of Extracts**
- The acetone extract was at pH 4.6 and was a muted, dark purple color.
- The water extract was at pH 6.7 and was a dark green-blue color.
- Both samples had similar chroma and lightness values.
- The methodology and pH contributed to the color differences[1].

**Color Change with pH**
- Both appeared pink when diluted with pH 1.0 buffer
- Both appeared blue-grey when diluted with pH 4.5 buffer.

**Total Anthocyanin Content**
- The water extract had 3.3 times higher total anthocyanin content than the acetone extract.

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>Total Anthocyanin Content (mg Delphinidin-3-glucoside equivalents/gram dried BPF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>4.55 ± 0.07</td>
</tr>
<tr>
<td>Water</td>
<td>15.20 ± 0.10</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**
- Hot water soaking worked efficiently as a method to extract pigments from dried BPF leaves.
- The pH, color characteristics, and total anthocyanin content of BPF pigment extracts differed between the two extracts.
- These results suggest that water extraction could be used to obtain blue pigment from butterfly pea flowers for use in food.
- Future research should look to optimize hot water extraction for development of an environmentally friendly procedure.

**BIBLIOGRAPHY**

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**Figure 2:** Extracts after dilution with water, pH 1.0 buffer, pH 4.5 buffer (left to right).

**Figure 3:** Color wheel graph of acetone (A) and water (W) extracts of dried BPF.

**Figure 4:** Absorbance Spectra and lambda max for dried BPF extracts.

**Table 1:** CIE L\*a\*b\* color characteristics for BPF extracts.

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>CIE L*a*b*</th>
<th>h (°)</th>
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</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>70.47</td>
<td>11.60</td>
</tr>
<tr>
<td>Water</td>
<td>68.84</td>
<td>13.14</td>
</tr>
</tbody>
</table>

**Table 2:** Anthocyanin content of dried BPF powder (n=3).

- Acetone: 4.55 ± 0.07 mg Delphinidin-3-glucoside equivalents/gram dried BPF
- Water: 15.20 ± 0.10 mg Delphinidin-3-glucoside equivalents/gram dried BPF

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