Lutein Content Analysis in Green and Red Spinach 
(*Amaranthus tricolor* L.) by Visible Spectrophotometry

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**Abstract**

Lutein is a yellow carotenoid compounds that is useful to protect the eyes from macular degeneration disease (AMD). It is found in vegetables such as spinach (*Amaranthus tricolor* L.). There are two types of spinach that had not been known how much the lutein content are. In this research, green and red spinach were macerated with aceton followed by extraction using diethyl ether. The absorption of the solution was measured in 445 nm. The content of lutein in green and red spinach are 292 mg/kg and 385 mg/kg with relative standard deviation of 3.07% and 2.03%.

**Key words**: Lutein, green spinach, red spinach, extraction, visible spectrophotometry

**Introduction**

Lutein is one of the carotenoids in eye lens. It is an antioxidant that can reduce the UV effect for eyes and as preventive for Aged-related Macular Degeneration (AMD) disease (Winarsi 2007). Many research were done to prove that conclusion, one of them was primate that has similar macular with man. After exposure of blue ray laser, the retina of primate that had no adequate lutein intake was more damage.

Lutein was found in the vegetables such as spinach, broccoli, zea mays, and yellow egg. According to Jean Mayer from US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, it was found of 6603µg/100 g in fresh spinach (Alisa 2008).

There are two kind of spinach (*Amaranthus sp*) that are wild spinach and cultivated spinach. Spinach that is often consumed were cultivated spinach that comprises of green and red spinach (*Amaranthus tricolor* L.)(Rukmana 1994). In this research lutein content analysis from green and red spinach was performed using spectrophotometry. Lutein has conjugated polyene so can absorb visible light. Green and
red spinach was macerated followed by partition step using Rodriguez-Amaya et al methods by modification.

**Methodology**

**Material, Chemicalia and instrumentation**

Lutein working standart, Green and red spinach (30 days of ages) were obtained from Cimanggu. Determination of the samples species name and their lose on drying was performed. Spectroscopic analysis was carried out on Shimadzu 1601 double beam UV/Visible spectrophotometer.

**Lutein extraction from green and red spinach.**

About 1 g of fresh spinach was cut into small pieces, then macerated with 30 ml of acetone that contained 0.1 mg of magnesium carbonate in a glass beaker. This step was performed during 16 hours with stirring. The residue was remacerated using 20 ml of acetone until the filtrate was clear. Final filtrate was tested using TLC method with n-hexane-chloroform-acetone (6:2:2) as mobil phase. The collected filtrate was added 10 mL of 50% KOH then heated on a hot plate for 10 minutes. The solution was put into a separating funnel and add 30 mL of diethyl ether. Added slowly 20 mL of distilled water. Shake for 2 minutes and allowed to stand. Water phase was discarded. Organic phase was washed with 20 ml of water two to three times as much. Diethyl ether phase was collected in 100 ml flask. Funnel rinsed with diethyl ether, diluted until 100 mL, then used as the test solution.

**Determination of maximum wavelength of lutein**

Absorbtion spectrum of working standard solution of lutein (100 ppm in diethyl ether) were recorded over the wavelength range of 300 to 700 nm against solvent blank, in quartz cuvetts with 1 cm diameters.

**Calibration curve**
Preparation of calibration curve was performed by measuring the absorption of working standard solution of 1.6 bpj; 2.3 bpj; 3 bpj; 3.7 bpj; 4.4 bpj; 5.1 bpj; and 5.8 bpj in 445 nm.

**Identification of lutein in red and green spinach**
Absorption spectrum of sample solution test were recorded over the wavelength range of 300 to 700 nm against solvent blank, in quartz cuvetts with 1 cm diameters.

**Determination of lutein content in green and red spinach**
The absorption of sample solution test was measured in 445 nm against diethyl ether as blank.

**Results and discussion**
Report from research Center for Biology, Indonesian Institute of Sciences mentioned that green and red spinach had the same species name, that is *Amaranthus tricolor* L. The lose on drying of green and red spinach are 81.78 % and 88.78% respectively. The spectrum of lutein standar solution was showed in Fig.1.

![Fig. 1. Absorption spectra of lutein standar solution in diethyl ether](image)

Determination of maximum wavelength was performed to choose the wavelength where the absorption of both standard solution and samples were measured. Fig 1. shows that
the maximum wavelength of lutein were 473, 445 and 331.5 nm. The highest absorption is in 445 nm. It is agree with the literature (Rodriguez 2001). Calibration curve of lutein that performed using seven different concentration of lutein showed that the equation of regression line was $y = 0.0421 + 0.1246x$ with correlation coefficient of 0.9998. This equation was used to calculate lutein content.

**Qualitative analysis**

Extraction methods in this study was performed using methods according to Rodriguez-Amaya by modification. According them, no need KOH added. KOH was added to mase rate to deliberate the chlorophyl of the spinach. Chlorophyl interfered the lutein absorption. The spectrum of samples without KOH was shown in Fig 2.

Fig 2. Absorption spectra of the samples without KOH

After KOH adding no more chlorophyl interference as shown in Fig. 3 and Fig. 4.

Fig 3. Absorption spectra of lutein (from green spinach) in diethyl ether

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Both of the samples had the maximum of the wavelength of about 445 nm.

Using calibration curve, the result of lutein content analysis can be seen in Table 1 and 2.

Table 1. The result of determination of lutein in green spinach

<table>
<thead>
<tr>
<th>Sample weight (g)</th>
<th>Absorption</th>
<th>Concentration of the sample solution (bpi)</th>
<th>Content of lutein against wet weight (%)</th>
<th>Content of lutein against dry weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0317</td>
<td>0.4102</td>
<td>2.9542</td>
<td>0.0286</td>
<td>0.1542</td>
</tr>
<tr>
<td>1.0496</td>
<td>0.4342</td>
<td>3.1469</td>
<td>0.0300</td>
<td>0.1615</td>
</tr>
<tr>
<td>1.0701</td>
<td>0.4207</td>
<td>3.0385</td>
<td>0.0284</td>
<td>0.1529</td>
</tr>
<tr>
<td>1.0373</td>
<td>0.4139</td>
<td>2.9839</td>
<td>0.0288</td>
<td>0.1550</td>
</tr>
<tr>
<td>1.0387</td>
<td>0.4354</td>
<td>3.1565</td>
<td>0.0304</td>
<td>0.1637</td>
</tr>
</tbody>
</table>

Table 1. The result of determination of lutein in red spinach

<table>
<thead>
<tr>
<th>Sample weight (g)</th>
<th>Absorption</th>
<th>Concentration of the sample solution (bpi)</th>
<th>Content of lutein against wet weight (%)</th>
<th>Content of lutein against dry weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0461</td>
<td>0.5390</td>
<td>3.9880</td>
<td>0.0381</td>
<td>0.3399</td>
</tr>
<tr>
<td>1.0513</td>
<td>0.5514</td>
<td>4.0875</td>
<td>0.0389</td>
<td>0.3466</td>
</tr>
<tr>
<td>1.0607</td>
<td>0.5347</td>
<td>3.9534</td>
<td>0.0373</td>
<td>0.3323</td>
</tr>
<tr>
<td>1.0498</td>
<td>0.5489</td>
<td>4.0674</td>
<td>0.0387</td>
<td>0.3454</td>
</tr>
<tr>
<td>1.0374</td>
<td>0.5502</td>
<td>4.0778</td>
<td>0.0393</td>
<td>0.3504</td>
</tr>
</tbody>
</table>

Extraction of lutein was performed using acetone as solvent. Aceton was the good solvent for carotenoid extraction. In order to protect the lutein from light, the Erlenmeyer
was wrapped. The residue of remasetad was tested by TLC methods using n-hexsane-chloroform-aceton (6:2:2) as mobil phase to determine that extraction of lutein was completed.

The result of lutein content in green and red spinach agains wet weight are 292 mg/kg (RSD of 3.07 %) and 385 mg/kg (RSD of 2.03), that fulfill the requirement for 100 mg/kg of analyts, that is 5%. It means that visible spectrophotometry is a precision method for determination of lutein in green and red spinach. Based on t test calculation to lutein content agains dry weight, the t value was 138.4915 (more than t table 2.306 (df = 8, p= 0,05). It means that there is a distinction of lutein content between green and red spinach.

**Conclusion**

Visible spectrophotometry was a precision methods for lutein determination in green and red spinach.

There are distiction content of lutein between green and red spinach. The average lutein content in green and red spinach are 292 mg/kg and 385 mg/kg respectively.

**Acknowledgement**

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**References**


Alisa P, Helen, R, Elizabeth, JJ. Xanthophyll (Lutein, Zeaxanthin) content in fruits, vegetables and egg product.2008

