Extraction and quantification of lutein from sweet potato leaves (Ipomoea batatas)

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INTRODUCTION

Carotenoids are yellow, red, or orange pigments which are spread in both plant and animal world. They came with green chlorophyll in leaves and herbs and are present in many flowers, fruits, seeds, or roots. They are fat-soluble nutrients and are categorized as xanthophylls or carotenes based on their chemical structure. These are regarded as important antioxidants, naturally present and have broad range of application in human health. The pigment properties of the carotenoids have been used for some wide range of application in the feed and food industries. Carotenoids are naturally present in edible green leaves, fruits, and flowers. Nowadays, attention is being given to utilization of plant sources for providing nutritional and pharmaceutical benefits to humans.

Green leafy vegetables are important sources of vitamins and minerals and also have wide range of health benefits. They are good sources of carotenoids, particularly lutein. Lutein belongs to the xanthophylls family of carotenoids which decreases the chances for eye diseases such as age-related macular degeneration, protects the skin, reduces cardiovascular problems, and aging and is being recommended for human, food and feed uses. It has a considerable market size as a feed additive.

Lutein (β, ε-Carotene-3, 3’ diol) is a naturally occurring derivative of hydrocarbon carotenoids. It is a fat-soluble pigment found in various fruits and vegetables and egg yolk. Lutein has important applications in better vision and protection of eye from harmful UV light. It is able to quench singlet oxygen that can damage DNA. During inflammation, lutein is depleted in immune tissues and the depletion level is dependent on dietary levels of lutein. There are various uses of lutein which include pigmentation of animal tissues and animal-based products, cosmetics and drugs, coloration of food, prevention of eye diseases such as age-related macular degeneration. Lutein is currently being important as a nutraceutical compound. Marigold flowers are important sources of lutein due to their high lutein content (4500 mg/lb). From a multinational study of carotenoids lutein levels in human, milk was found to be in the range of 14.8–43.8 mcg lutein/L. Infant formula follows of milk for toddlers and young children were formulated to

ABSTRACT

Introduction: Sweet potato leaves are discarded as waste after harvesting of tubers, which has high level of carotenoids, particularly lutein. Lutein is an important carotenoid commonly found in dark green leafy vegetables, fruits, as well as in corn and egg yolk. Since lutein cannot be synthesized in the body, it is supplemented through diet as the main source. It is absorbed and concentrated in part of the eye which protects eye health by filtering out harmful light with its antioxidant properties. The average intake of lutein was estimated to be 1.78 mg/person per day. The intake of lutein through diets is important in the prevention of diseases such as cancer and cardiovascular diseases and also in the prevention of age-related macular degeneration. Objective: The aim of this study is to extract lutein from sweet potato leaves and determine its yield and to enhance the bioavailability. This study focuses on the effect of different types of processing conditions on the lutein content to determine the most desirable source which offers high yield and affordability.

KEY WORDS: Age-related macular degeneration, Antioxidant, Bioavailability, Lutein
provide a lutein source and a validated method was needed to confirm the fortification level. In green plants such as broccoli, green beans, green peas, lima beans, spinach, collards, mustard greens, turnip greens, cabbage, kale, alfalfa, kiwi, and honeydew, lutein is present in the form of a free, non-esterified form. Lutein extraction from green plants and vegetables may be essential because it eliminates the additional step of saponification to obtain the free lutein. However, in the past, the purification and isolation of lutein from green leafy plants has not been economical because many expensive and time-consuming steps have been involved to separate the lutein from the other compounds present.

Currently, lutein is included in many dietary supplements present because of its important role in functioning and health benefits for the eyes and to treat and prevent some type of cancers. Nowadays, lutein is mainly extracted from marigold flowers, one of the best natural sources of xanthophylls that use a pre-treatment of the raw material to enhance both the yield and the quality of the olearoerin. Therefore, the need for finding an alternative, relatively less expensive sources of lutein becomes important.

Sweet potato or Ipomoea batatas originated from the Northwest region of South America has been found worldwide due to its high yield potential and more adaptability. These plants are relatively easy to grow and are used mainly for their storage roots. The leaves are discarded as waste or being made into animal feeds after harvesting period. The nutritional value of leaves is gaining importance, as the knowledge between human health and diet increases. Sweet potato leaves with their high nutritional value and antioxidant property have become an excellent green leafy vegetable. Along with this, this crop has higher tolerance to pest, moisture, and diseases and can be planted several times a year. Sweet potato leaves have been studied as a suitable source of relatively cheap natural dye (yellow) that can be used to replace the currently used synthetic dye. These leaves have benefits over the other sources because of their low economic value and the suitability to provide long-term supply.

A study by Ishiguro and Yoshimoto showed that the sweet potato leaves contain high lutein content compared to other green leafy vegetables. Hence, these leaves are further studied to be a good source of lutein. The availability of lutein pigment in a plant is affected by plant variety, type and growth stage of the leaf. The main objectives of the present study are to extract lutein from sweet potato leaves, comparative study of the yield of lutein from fresh, frozen, and dried leaf samples and to estimate the yield of lutein. This study also focuses on the determination of the most suitable processing condition which shows the highest yield of lutein content.

**MATERIALS AND METHODS**

Fresh sweet potato leaves were collected from the farm at Thrissur, Kerala. Raw materials were divided into three groups, cut into small pieces, rinsed with potable water, and shade dried to remove the surface moisture before subjected to different processing conditions.

**Analytical Procedure**

Among the three lots of fresh sample, one was kept as unprocessed fresh leaf sample (control). The second lot was dried using a tray dryer (6–10 h at 60°C) till it attains the equilibrium moisture content and dried samples were stored in airtight container. The third lot of sample was frozen at −18°C and stored in a deep freezer before extraction. Each of the three samples was taken separately and subjected to the following extraction method. About 2 g of each leaf sample was ground using a mortar and pestle separately. Then, the samples were treated with acetone (80 ml) in screw-capped containers for extraction. This mixture was shaken for 1 h under dark condition to inhibit decomposition of lutein in a mechanical shaker and filtered using Whatman No 1 filter paper. The filtrate was again subjected to extraction process. Then, it was evaporated using a rotary evaporator. Saponification was done by dissolving the extract obtained in 10% potassium hydroxide dissolved in methanol. These samples were shaken in dark for overnight. The unsaponifiable matters were treated with hexane: ethyl ether (1:1) solution in a separating funnel for extracting lutein. This was done under dark condition to lessen degradation. The washing of the aqueous phase (lower) was done with the hexane/ethyl ether solution for re-extraction until the aqueous phase (lower) was colorless. All the hexane: ethyl ether extracts that contained the lutein carotenoid were combined and evaporated. The extract was dissolved in the ethanol and then ethanol was removed using vacuum distillation. These two steps were repeated for 3 times to remove all the traces of the mixture of hexane: ethyl ether solution. The lutein extract was stored at −20°C followed by quantification using high-performance liquid chromatography (HPLC).

Carotenoid analysis was done using Shimadzu LC solution - HPLC equipped with a photodiode array detector. Samples were analyzed by HPLC to identify lutein components in the samples. The separation process was carried out isocratically using 100% methanol (MeOH) as the mobile phase with 0.5 ml/min flow rate and a temperature of 16°C. The sample injection volume was 20 µl (manually) and the chromatograms were processed at wavelengths...
of 240 nm. The total separation time for each sample was 30 min. The carotenoid identification was done by comparison of retention time (tR) with those of the standard compounds. Using the chromatograms, the sample peak area units were compared with the reference standard peak area units of lutein, and the percentage of free lutein against the lutein reference standard solution was determined. The lutein content was determined using the following formula:

\[
\text{Lutein content (\%) = \frac{A \times \text{Response factor}}{S \times \text{Dilution factor}} \times 100}
\]

Where, A = Area and S = Sample weight

RESULTS AND DISCUSSION

Analysis of Lutein from the Sweet Potato Leaf Extract

For quantification of lutein in the extracted samples, HPLC method was used. Calibration of lutein standard samples was done with different solutions of lutein standard (known concentrations) to determine the linearity between lutein peak areas against injection mass concentrations.

The lutein content of all the three extracts was quantified and presented in Table 1. In the HPLC analysis, all the samples detected the peak at a tR between 5.9 and 6.3 min. To confirm the presence of other compounds, the sample was injected at various wavelengths and it was found that there were no considerable disturbances in the lutein region was observed.

It is observed from Figure 1 that HPLC analysis of lutein for the unprocessed fresh leaf sample was identified at tR of 5.93 min in comparison with the standard method. The peak area corresponding to its tR is shown in the table below.

Further, analysis was conducted for the dried leaf sample extract. In this case, the lutein content was identified at a retention time (tR) of 6.3 min with an area value less than that of the fresh leaf sample. The HPLC profile is shown in Figure 2.

The frozen samples showed a peak value at the tR at 6.247 min and the HPLC results of the frozen leaf sample extract for the analysis of lutein content are mentioned in Figure 3. The result was compared with the other two values and it is observed that the values are lower than that of the dried leaf sample.

Using the equation for lutein content, the amount of lutein present in each leaf sample was determined. The results are shown in Table 1.

It is clearly evident from the results that the processing condition invariably affects the lutein content of sweet potato leaves.
Table 1: Quantification of lutein

<table>
<thead>
<tr>
<th>Sample no</th>
<th>Lutein yield (%)</th>
<th>Lutein content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>44.23</td>
<td>442.3</td>
</tr>
<tr>
<td>Sample 2</td>
<td>1.47</td>
<td>14.7</td>
</tr>
<tr>
<td>Sample 3</td>
<td>17.95</td>
<td>179.5</td>
</tr>
</tbody>
</table>

potato leaves. The results showed that the fresh sweet potato leaves have higher lutein content (44.23%) compared to dried and frozen leaf samples. The frozen leaf sample has 17.95% of lutein which is higher than that of the dried sample. The lutein content in unprocessed, dried, and frozen leaf samples is 442.3, 14.7, and 179.5 mg/g, respectively. The above results show that fresh leaves contain more lutein content than frozen and dried samples.

**CONCLUSION**

Sweet potato leaves are good source of lutein. Its use as a green leafy vegetable is novel in most of the countries. These leaves in both their processed and unprocessed forms have high lutein content. They have also possessed high retinol equivalents. However, household processing and preparation procedures, such as freezing and drying, will affect the lutein content. This study was carried out to determine the lutein content in both unprocessed and processed sweet potato leaves.

In the HPLC analysis, lutein was eluted between 5.9 and 6.3 min. Lutein was identified by comparing the tR with that of the standard. The results from the HPLC chromatograms showed that different processing conditions will affect the lutein content. The results showed that sample 1, i.e., fresh sweet potato leaves have higher lutein content (44.23%) compared to dried and frozen leaf samples. The frozen leaf sample has 17.95% of lutein which is higher than that of the dried sample. The lutein content in unprocessed, dried, and frozen leaf samples is 442.3, 14.7, and 179.5 mg/g, respectively. Fresh leaves contain more lutein content than the processed leaves samples.

**REFERENCES**


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