Chapter 2

Biochemical changes and antioxidant activity of elephant-foot yam corm during development
Introduction

The corm of elephant-foot yam is used as vegetable and also as a major ingredient in various ayurvedic preparations. It is used in the treatment of piles, dysentery, acute rheumatism, asthma and hemorrhoids. It has revitalizing effect on acidity, heartburn, pain in abdomen and in intestinal worms. It exhibits various biofunctional properties like analgesic, antioxidant, immunomodulatory activity etc. The components of corm impart such wide array of functions to the elephant-foot yam. Harvesting of corm at peak accumulation of its bioactive constituents during its growth and development is of vital importance in relation to health benefits.

Participation of bioactive compounds in an array teleological functions as precursors in imparting characteristic flavour, color, defense intermediaries and health benefiting factors in fruits, vegetables, and rhizomes were well documented (Tholl, 2006). Temporal variation in the concentration of bioactive molecules is regulated by a complex interaction between intrinsic plant factors and external factors, both abiotic and biotic (Herms and Mattson, 1992; Beckman, 2000; Booij-James et al., 2000). Interestingly, they showed site and cells specificity in accumulation, as a function of maturity (Kause et al., 1999; Samanani, 2002). Lack of such studies in elephant-foot yam is apparent despite its pharmaceutical importance.

Coordinated biochemical alterations during development and growth elephant-foot yam determine the quality of corms in terms of maturity and peak accumulation of bioactive compounds. Extensive review of literature showed lack of any maturity index for harvest. Hence the current study was conducted to know the accumulation pattern of phenolics along with changes in solubles and storage components as a function of nutraceutical maturity. A time course study of these changes from the time
of planting to harvest, which ranged from 0 to 270 days at a time interval of 30 days, was conducted.

**Materials and Methods**

**Sample collection**

Corms of elephant-foot yam were collected from commercial plot near Ambalavayal, Wayanad (District), Kerala, India. The first sampling time (90 days after planting) was conducted when the corms initiated their growth. Subsequently, the samples were collected at 120, 150, 180, 210, 240 and 270 days after planting. Each sample was prepared from corms obtained from five plants that were harvested randomly from the field. All the biochemical analysis and other experiments were carried out in triplicates.

**Sample preparation**

Corms were sliced, pulped and homogenized to get the sample to determine pH, titrable acidity, total soluble solids (TSS), sugar content, total phenolic, flavonoid and carotenoid content and antioxidant activity.

**pH, total soluble solids, titrable acidity, moisture content and texture**

pH of the pulp was measured using Control Dynamics pH meter calibrated with standard buffer pH 7. The total soluble solids (TSS) were determined by a digital refractometer (ATAGO RX-5000, ATAGO, Japan) calibrated with distilled water. Titrable acidity was determined by AOAC (1990) method. Moisture content was estimated by gravimetric method. Texture (Penetration, compression and shear) was measured by using Instron Universal Testing machine, INSTRON Model-4301, U.K.
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**Total carotenoid content**

Total carotenoid content estimated by using the method described by Ranganna (1986).

**Total phenolic content**

The total phenolic content in the pulp was determined with the modified method of Taga et al. (1984). In brief, 100 µL of sample was mixed with 2 mL of 2% aqueous sodium carbonate solution. After 3 min, 100 µL of 50% Folin-Ciocalteau phenol reagent was added to the mixture. After 30 min of incubation at room temperature, absorbance was measured at 750 nm against a blank. Total phenolic content was calculated on the basis of the standard curve of gallic acid.

**Total flavonoid content**

The TFC of the pulp was determined by aluminum chloride colorimetric method (Esmaeili and Sonboli, 2010; Gulcin et al., 2010) 100 µL of sample was placed in a 10 mL volumetric flask and then 5 mL of distilled water added followed by 0.3 mL of 5% NaNO₂. After 5 min, 0.6 mL of 10% AlCl₃ was added. After 5 min, 2 mL of 1M NaOH was added and volume made up with distilled water. The solution was mixed and absorbance measured at 510 nm using UV–visible spectrophotometer. TFC was calculated from calibration curve of catechin.

**DPPH assay**

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity was determined according to the method described earlier (Blois, 1958; Bondet et al., 1997; Moon and Terao, 1998). The test samples (100 µl) were mixed with 0.9 ml of Tris-HCl buffer (pH 7.4) to which 1 ml of DPPH (500 µM in ethanol) was added. The
mixture was shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm in a UV-Visible Spectrophotometer (UV-160A, Shimadzu co. Japan). The radical scavenging activity was measured as a decrease in the absorbance of DPPH. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

\[
\text{Antioxidant activity (\%)} = \left(1 - \frac{A_{\text{sample (517 nm)}}}{A_{\text{control (517 nm)}}}\right) \times 100
\]

**Total reducing power**

The reducing power was quantified by the method described earlier by Yen and Chen (1995) with minor modifications. Reaction mixture, containing test samples at different concentrations (10-100 µl) in phosphate buffer (0.2 M, pH 6.6), was incubated with potassium ferricyanide (1 % w/v) at 50°C for 20 min. The reaction was terminated by the addition of TCA solution (10 % w/v) and the mixture was centrifuged at 3000 rpm for 20 min. The supernatant was mixed with distilled water and ferric chloride (0.1 % w/v) solution and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

**Results and discussion**

**Developmental stages and yield of corm**

Accumulation of bioactive components and their antioxidant activity was investigated during developmental stages of elephant-foot yam corm from 90 to 270 days. Four distinct phases of growth and development in corm were defined, namely: (1) vegetative growth phase; (2) corm initiation and growth phase; (3) maturation phase; and (4) senescence phase (Table 2.1).
Table 2.1 Growth phases of elephant-foot yam corm

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Parameter (fresh pulp)</th>
<th>Duration (days from planting)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vegetative growth phase</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>Corm initiation and growth phase</td>
<td>90-180</td>
</tr>
<tr>
<td>3</td>
<td>Maturation phase</td>
<td>180-210</td>
</tr>
<tr>
<td>4</td>
<td>Senescence phase</td>
<td>&gt;210</td>
</tr>
</tbody>
</table>

Active vegetative growth extends up to 90 days from planting. The events were evident by the formation of a few pair of leaves. Initiation of corm was observed little earlier to 90 days from planting. The yield of corms increased rapidly after 90 days and highest yield was recorded on 210 days after planting (Fig 2.1). A conspicuous drying of leaves 210 days gives a visual marker for maturity of corm. The maturation of corm was characterized by the increase in size, weight and yield that may be due to rapid accumulation of bioactive and storage components like starch, proteins and phenolics.

**Fig 2.1 Yield of corm during developmental stages**

Each value is a mean of three different observations
Phenolic, flavonoid and carotenoid content
Accumulation of phenolics occurred immediately after the initiation of corm formation, with an initial concentration of 26 mg/100 g and reached maximum (117 mg/100 g) at 210 days (Fig 2.2). Flavonoid (Fig 2.3) and carotenoids (Fig 2.4) accumulation followed similar pattern and reached maximum (69.37 mg/100gm for flavonoids and 601µg/100gm for carotenoids) at 210 days. High content of phenolics, flavonoids and carotenoids may be an essential component for defense against various pathogens that are constantly challenging the underground corm. Phenolics from various plant sources and their contribution to antimicrobial and other biochemical responses are well documented (Benner, 1993; Bennett and Wallsgrove, 1994). It appears that peak accumulation of bioactive constituents on 210 days may herald the onset of senescence. Later decrease in above may be attributed to strengthening of the plant cell walls by polymerization into lignans and lignins (Randhir and Shetty, 2005). Therefore, the synthesis and accumulation pattern of bioactive constituents may be used as an indicator to differentiate the physiological maturity and quality of elephant-foot yam corm.

Fig 2.2 Changes in phenolic content of corm during development
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**Fig 2.3** Changes in flavonoid content of corm during development

![Flavonoid content graph](image1)

**Fig 2.4** Changes in carotenoid content of corm during development

![Carotenoid content graph](image2)
Antioxidant activity

Antioxidant activity measured by DPPH assay (Fig 2.5) and total reducing power (Fig 2.6) revealed that there was gradual increase in DPPH radical scavenging activity and total reducing power and reached maximum at 210 days. This may be attributed to the high content of phenols, flavonoids and carotenoid content at 210 days. An increase of antioxidant activity associated with accumulation of bioactive compounds at 210 days could be a better method to determine the optimum nutraceutical maturity to harvest corm of elephant-foot yam, rather than conventional harvest from 240 to 270 days after planting.

Fig 2.5 Changes in DPPH radical scavenging activity of corm during development
The present study clearly indicated that the synthesis and accumulation pattern of active components served as bioactive markers to determine the nutraceutical maturity for harvest of the elephant-foot yam corms. The distinct patterns of these biochemical markers were associated with conspicuous display of drying and detachment of leaves from the corm. This provides a visual clue for maturation of corm. The various maturity indices at 210 days (Table 2.2) from planting were found to be optimum for harvest, compared with the conventional harvest ranges from 240 to 270 days.
Table 2.2 Physico-chemical parameters of corm of elephant-foot yam at nutraceutical maturity (210 days from planting)

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Parameter(fresh pulp)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>5.98</td>
</tr>
<tr>
<td>2</td>
<td>TSS (%)</td>
<td>5.58</td>
</tr>
<tr>
<td>3</td>
<td>Total Acidity (%)</td>
<td>0.27</td>
</tr>
<tr>
<td>4</td>
<td>Moisture content (%)</td>
<td>76.82</td>
</tr>
<tr>
<td>5</td>
<td>Pulp/Peel ratio</td>
<td>18.86</td>
</tr>
<tr>
<td>6</td>
<td>Penetration(Newton)</td>
<td>11.47</td>
</tr>
<tr>
<td>7</td>
<td>Compression(Newton)</td>
<td>50.66</td>
</tr>
<tr>
<td>8</td>
<td>Shear(Newton)</td>
<td>8.01</td>
</tr>
<tr>
<td>9</td>
<td>Total Carotenoids(µg/100g)</td>
<td>601.69</td>
</tr>
<tr>
<td>10</td>
<td>Total Phenols(mg/100g)</td>
<td>117</td>
</tr>
<tr>
<td>11</td>
<td>Total Flavonoids(mg/100g)</td>
<td>69.37</td>
</tr>
</tbody>
</table>

Conclusion

For the first time, a new set of nutraceutical maturity indices for harvest of elephant-foot yam corm has been established, based on the synthesis and accumulation pattern of bioactive constituents during development of elephant-foot yam corm. Its maturation is also directly influenced by coordinated alterations of several biochemical factors. Interestingly, they demonstrated a distinct pattern of accumulation governed by the physiological maturity of the corm during development. The importance of these patterns highlighted in the present investigation which I believe has commercial application.