JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Accumulation of Bioactive Compounds during Growth and Development of Mango Ginger (*Curcuma amada* Roxb.) Rhizomes

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Accumulation of bioactive compounds and storage components during developmental stages of mango ginger (*Curcuma amada* Roxb.) rhizome was investigated from 60 to 240 days, as a function of physiological maturity. Four distinct developmental phases were defined, namely, vegetative phase (up to 60 days from planting), initiation and development phase (60–150 days), maturation phase (150–180 days), and senescence phase (180 days). Difurocumenonol, a bioactive terpenoid compound and phenolics were identified as biomarkers, to determine the optimum physiological maturity to harvest mango ginger rhizome. Accumulation of phenolics was observed in newly initiated rhizomes (after 60 days from planting). The phenolic content was high in mango ginger pulp compared to its juice. Newly initiated rhizome contained no difurocumenonol, and it was observed after 120 days after planting. Peak accumulation of phenolics, difurocumenonol, and total protein were noticed in 180 day old rhizome. Accordingly, the abundance of these components on 180 days was set as an optimum maturity standard for harvest of mango ginger rhizome, compared with a conventional harvest period that ranges from 200 to 240 days.

KEYWORDS: Antioxidant activity; bioactive compounds; *Curcuma amada* Roxb.; difurocumenonol; development; mango ginger; maturity; phenolics; proteins; rhizome

INTRODUCTION

The genus *Curcuma* belongs to the family Zingiberaceae comprising more than 80 species of rhizomatous herbs. They originated in the Indo-Malayan region and are distributed widely in the tropics from Asia to Africa and Australia (*1*). Mango ginger (*Curcuma amada* Roxb.) is a perennial herb, and its rhizomes are morphologically similar to ginger but impart mango flavor that has been attributed to volatile compounds like car-3-ene and *cis*-ocimene (*2*). Hence, the rhizomes are highly valued and extensively used in the preparation of pickles and salads. Though mango ginger rhizomes resemble ginger morphologically, they differ in biochemical composition including starch granules. Structure and size of mango ginger starch granules varies significantly from turmeric starch and ginger starch by the absence of fissures on the surface and also by its X-ray diffraction pattern. It occupies a unique position between turmeric and ginger starch (*3*).

Ayurveda, the oldest system of medicine in India, has given importance to mango ginger rhizome, as an appetizer, alexteric, antipyretic, aphrodisiac, and laxative. It is also used against biliousness, itching, skin diseases, bronchitis, asthma, hiccups, and inflammation due to injuries. According to the Unani system of medicine, it is used against inflammation in the mouth, ear, and gleet, ulcers on the male sex organs, scabies, lumbago, and stomatitis (*4–6*). Anti-inflammatory activity in acute and chronic albino rats was demonstrated by the ethyl alcohol extract of mango ginger (*7*). It has been reported to inhibit trypsin enzyme (*8*) and also found to induce hypotriglyceridemic activity in Triton-induced hyperlipidemic rats (*9, 10*). Recently, two new bioactive terpenoids, namely, difurocumenonol and amadannulen, were isolated and characterized from mango ginger rhizome, of which the former was found to be a major bioactive compound (*11*). They exhibited various bioactivities, namely, antibacterial activity, platelet-aggregation inhibitory activity, lipoxygenase-inhibitory activity, and cytotoxicity and antioxidant activities, namely, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, lipid-peroxidation inhibitory activity, metal chelating activity, and superoxide radical scavenging activity (*11,12*). Participation of bioactive compounds in an array of functions as precursors in imparting characteristic flavor, color, defense intermediaries, and health benefiting factors in fruits, vegetables, tubers, and rhizomes were well documented (*13*). Interestingly, they show site and cell type specificity in accumulation, as a function of maturity (*14, 15*). There is a worldwide interest in understanding functions of nutritional and nutraceutical components in fruits, vegetables, and spices, but underground storage tissue like rhizomes, tubers, and roots are relatively unexplored.

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10.1021/jf0715469 CCC: $37.00
Published on Web 08/23/2007

Mango ginger is one such vegetable, despite its pharmaceutical importance and exotic mango flavor.

Because coordinated biochemical alterations determine the quality of rhizomes during development in mango ginger plant, the present investigation was focused on elucidating the accumulation pattern of difurocumenonol, along with phenolics and soluble and storage components as a function of physiological maturity and harvest indices of the rhizome of mango ginger.

MATERIALS AND METHODS

Sample Collection. The rhizomes were planted in an experimental plot near Hassan, Karnataka, India, during June 2005. The first sampling time (60 days after planting) was conducted when the rhizomes initiated their growth. Subsequently, the samples were collected at 90, 120, 150, 180, 210, and 240 days after planting. Each sample was prepared from rhizomes obtained from five mango ginger plants that were harvested randomly from five different beds. All the biochemical analysis and other experiments were carried out in triplicate.

Chemical Composition of Mango Ginger. *Sample Preparation.* About 500 g of mango ginger rhizomes were sliced, homogenized, and squeezed in two-layered muslin cloth, to extract the complete juice. The juice was centrifuged at 8000 rpm for 20 min at 4 °C and used to determine pH, titrable acidity, total soluble solids (TSS), sugar content, protein content, phenolic content, and antioxidant activities.

The pulp (residue) left after the extraction of juice is still a rich source of bound phenolic compounds. Hence, the pulp was homogenized with 80% methanol to extract the phenolics completely. The extraction was repeated until it became colorless. The methanol extract was filtered and evaporated using a rotary evaporator. The extract was dissolved and diluted to a final volume of 100 mL with 80% methanol. The mixture was centrifuged at 8000 rpm at a refrigerated temperature (4 °C) for 20 min and used for determination of total phenolic content, DPPH radical scavenging activity, and total reducing power.

Extraction and Quantification of Difurocumenonol by HPLC. To study the accumulation and quantification of difurocumenonol, the fresh rhizomes (10 g) were homogenized with chloroform until they became colorless. The extract was filtered and concentrated using a rotary evaporator and freeze-dried before using the sample for HPLC analysis. Difurocumenonol (the isolated compound) and chloroform extracts obtained during different developmental stages were tested using a LC-10AT liquid chromatograph (Shimadzu, Singapore) equipped with 300 × 4.6 mm i.d., 5 μ, Thermo Hypersil C-18 column (Bellefonte, PA, U.S.A.). The gradient program used for the mobile phase was methanol/water, as follows: 0 min, 25:75 v/v; 5 min, 40:60 v/v; 10 min, 50:50 v/v; 20 min, 70:30 v/v; 40 min, 90:10 v/v; and 60 min, 100:0 v/v, with a flow rate of 1 mL/min. UV detection was carried out with a SPD-M10A VP diode array detector (Shimadzu, Singapore), operated at 240 nm.

Determination of Phenolics. The total phenolic content in mango ginger juice as well as pulp was determined with the modified method of Taga et al. (*16*). 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.

DPPH Radical Scavenging Activity. DPPH radical scavenging activity was determined according to the methods described previously (*17, 18*). The test samples (100 μL) were mixed with 0.8 mL of Tris-HCl buffer (pH 7.4) to which 1 mL of DPPH (250 μM in ethanol) was added. The mixture was shaken vigorously and kept for 30 min. Absorbance of the resulting solution was measured at 517 nm in a UV-160A spectrophotometer (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.

Total Reducing Power. The reducing power was quantified by the method described by Yen and Chen (*19*) with minor modifications. The reaction mixture, containing test samples (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 trichloroacetic acid (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.

Reducing Sugars, Total Sugars, and Total Protein Content. Reducing sugars and total sugars were determined by as described by Ranganna (*20*). The total protein content was determined by the Bradford method (*21*), using bovine serum albumin (BSA; Sigma Chemical, St. Louis, U.S.A.) as a standard protein.

pH, Titrable Acidity and Total Soluble Solids. pH of the fresh juice was measured using a pH meter calibrated with standard buffer at pH 7. Titrable acidity was determined by the AOAC (*22*) method. The TSS were determined by an RX-5000 digital refractometer (ATAGO, Japan) calibrated with distilled water. Mango ginger juice was passed through a filter paper (Whatman No. 1) using vacuum before analysis.

Statistical Analysis. The data were subjected to Duncan's multiple range test (DMRT) to determine significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

Developmental Stages and Yield of Mango Ginger. Synthesis and accumulation of bioactive compounds along with other soluble and storage components were investigated during developmental stages of mango ginger rhizome from 60 to 240 days. Four distinct phases of growth and development in mango ginger plant were defined, namely, (1) vegetative growth phase; (2) rhizome initiation and growth phase; (3) maturation phase; and (4) senescence phase (**Figure 1**). Active vegetative growth extends up to 60 days from planting. The events were evident by the formation of six to eight pairs of green leaves. Initiation of rhizomes was observed after 60 days from planting. The yield of rhizomes increased rapidly after 90 days, and the highest yield (1.95 kg/plant) was recorded 180 days after planting. A conspicuous drying of leaves after 150 days and falling of leaves after 180 days gives a visual marker for maturity of mango ginger rhizomes. The maturation of rhizomes 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. In contrast, total sugar and reducing sugar contents decreased. The decline in all these components after 180 days heralds the onset of the senescence phase.

Differentiation of Rhizome. The internal tissue of rhizome remained undistinguished as a white, juicy mass of cells until 90 days of planting (**Figure 2**). Differentiation of central, circular, yellow colored pith that was demarked from the surrounding white cortex tissue was observed after 120 days. The ratio between pith and cortex during the growth period remained constant and measured 1:1 (w/w). With the onset of maturity, a significant increase in pith (2:1, w/w) was observed. In addition, change in color of pith from yellow to greenish yellow was exhibited. Dispersion of yellow color to cortex region was also visible (**Figure 2**). Coincidently, these changes were in accordance with physiological maturity of rhizomes. Thus, they could be identified as visual maturity indices.

Accumulation of Difurocumenonol. Difurocumenonol (**Figure 3**), a terpenoid compound, has been reported to be an antimicrobial compound against a wide range of microbes (*11*). Accumulation of difurocumenonol in mango ginger rhizomes during developmental stages (60–240 days) was carried out using HPLC. Newly initiated rhizomes contained no difurocumenonol, and it was first observed in 120 day old rhizomes. The highest concentration of difurocumenonol (34 mg/100 g) was noticed in 180 day old rhizomes (**Figure 3**).The synthesis and accumulation of this compound in the developing rhizome

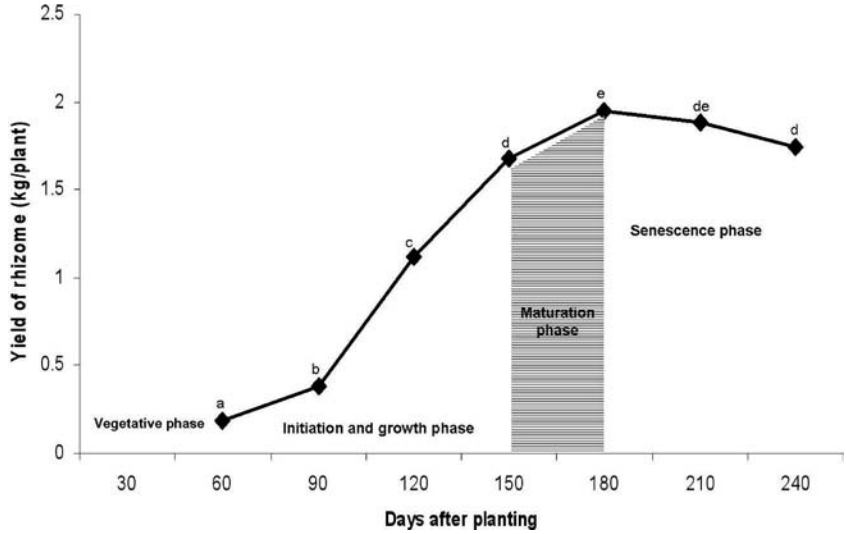

Figure 1. Yield of mango ginger rhizomes during different developmental stages. Each value is a mean of three different observations. Values shown by different letters for each line are significantly different at $p < 0.05$.

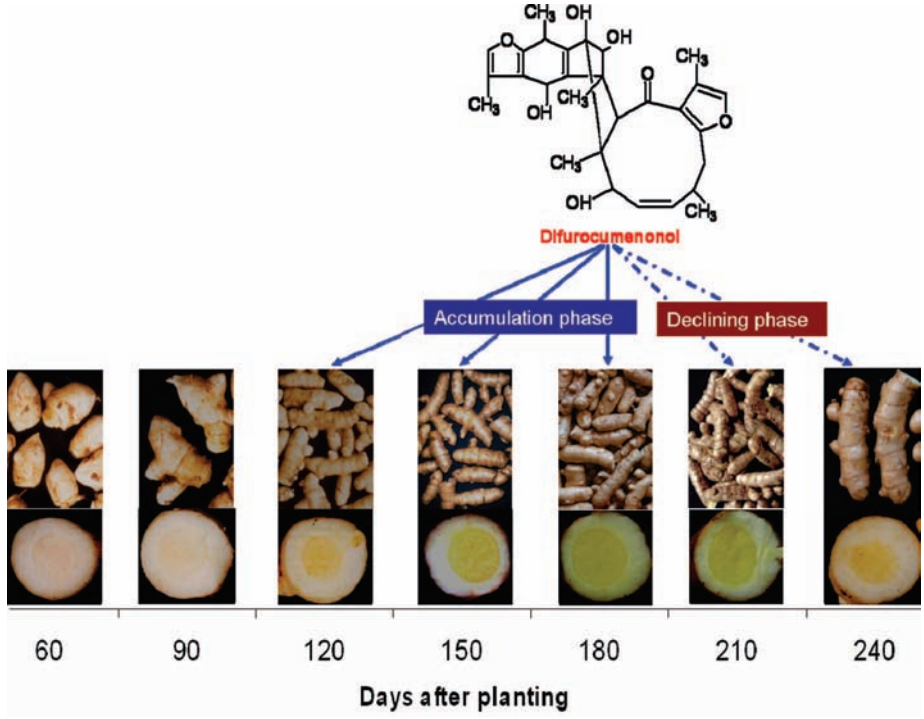

Figure 2. Different stages of growth and development of mango ginger rhizomes with transverse section showing internal color.

during growth and maturation of rhizome is essential to counteract bacteria and fungi. The terpenoids play a major role in contributing to flavor components and defense intermediates in plants (*23*). Participation of difurocumenonol in synthesis of flavor components in mango ginger may be responsible for its decrease in concentration during senescence. The tissue-specific biosynthesis and accumulation of difurocumenonol has yet to be identified. It is interesting to note that the pattern of synthesis, accumulation and degradation of difurocumenonol is in accordance with growth, maturation, and senescence of the rhizome. Hence, it has been identified as a nutraceutical marker to determine the physiological maturity of rhizome and as a harvest index.

Physiological Role of Difurocumenonol. It may be a biological inevitability for mango ginger rhizome to develop compounds of multifunctional activity to counteract the diversified underground abiotic and biotic challenges. Difurocumenonol proved to be one such compound with multifunctional properties found in mango ginger. The antimicrobial nature of difurocumenonol (*11*) can effectively thwart the constant challenges posed by underground pathogens. In addition, it possesses a wide range of antioxidant activities and other bioactive properties (*24*). High antioxidant property provides stability against auto-oxidation. Thus, difurocumenonol ensures persistent and prolonged antimicrobial activity during growth, senescence, and the dormancy period of rhizome. High lipid peroxidation inhibitory activity and metal chelating activity of difurocumenonol (*24*) may act as competitive inhibitor for sprouting, as a result of its high oxygen demand. Thus, it may offer physiological protection to tide over dormancy. The terpenoids play a major role in contributing to flavor components and defense intermediates in plants (*23*). Difurocumenonol being a terpenoid compound, its derivatives may participate as metabolic intermediaries in synthesis of flavor during distress storage conditions

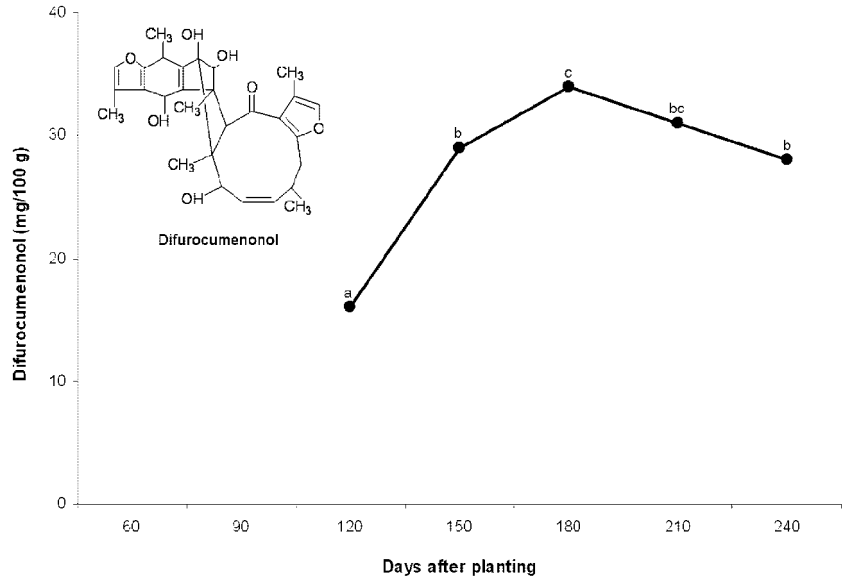

Figure 3. Concentration of difurocumenonol (structure inset) in mango ginger rhizomes during different developmental stages. Each value is a mean of three different observations. Values shown by different letters for each line are significantly different at $p < 0.05$.

and during senescence depending upon the physiological needs. Thus, it may be responsible for the persistence of mango flavor in rhizome even after shriveling beyond the level of commercial acceptability. The role of difurocumenonol derivatives in various other physiological activities is interesting and worth investigating.

Phenolic Content. The phenolic content was quantified in mango ginger at different developmental phases. Accumulation of phenolics occurred immediately after the initiation of rhizome formation, with an initial concentration of 122 mg/100 g and 26 mg/100 g in pulp and juice, respectively (**Figure 4A**).The abundance of phenolics on the 120th day in juice and 180th day in pulp is attributed to increase in weight of rhizomes during the growth and maturation phase of the rhizome. The concentration of phenolics in pulp was 10 times higher than that of phenolics in juice, at all the stages of development of rhizome. High content of phenolics may be an essential component for defense against various pathogens that are constantly challenging the underground rhizome, as evident from the antimicrobial activity of mango ginger extracts containing phenolics with other bioactive compounds such as difurocumenonol and amadannulen that were isolated from mango ginger rhizome (*11, 12*). Phenolics from various plant sources and their contribution to antimicrobial and other biochemical responses are well documented (*25, 26*). It appears that peak accumulation of phenolics in pulp on the 180th day may herald the onset of senescence in mango ginger rhizome. Decrease in phenolics may be attributed to strengthening of the plant cell walls by polymerization into lignans and lignins (*27*). Therefore, the synthesis and accumulation pattern of phenolics may be used as an indicator to differentiate the physiological maturity and quality of rhizome in mango ginger.

Antioxidant Activity. *DPPH Radical Scavenging Activity.* Mango ginger juice exhibited a gradual decrease in DPPH radical scavenging activity until 240 days during developmental stages. In contrast, mango ginger pulp showed a gradual increase in DPPH radical scavenging activity, which was highest on 180 days of development and decreased thereafter until 240 days (**Figure 4B**). The DPPH radical scavenging activity of pulp has been attributed to the concentration of difurocumenonol and total phenolics (*24*). Antioxidant activity of amadannulen and mango ginger extracts and phenolics were reported in mango ginger and also in other vegetables (*12, 28, 29*). In addition, terpenoids also have medicinal properties such as anticarcinogenic, antimalarial, antiulcer, hepatoprotective, antioxidant, and antimicrobial activity (*11, 30*). An increase of antioxidant activity associated with accumulation of bioactive compounds like phenolics and difurocumenonol at 180 days could be a better method to determine the optimum physiological maturity to harvest mango ginger rhizomes rather than conventional harvest from 200 to 240 days after planting.

Total Reducing Power. The reducing power of pulp increased with rate of growth and development and recorded the highest concentration after 180 days. There was an initial spurt in reducing power of mango ginger juice up to 90 days and a decrease thereafter (**Figure 4C**). The reducing capacity of samples from the ferricyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity (*31*). The reducing power of mango ginger pulp was almost 10 times higher than that of juice. This may be attributed to the presence of a high concentration of difurocumenonol, amadannulen, and phenolics in the pulp of mango ginger as reported earlier by the author (*11, 12*).

Total Sugars and Reducing Sugars Total Protein Content. Total sugars and reducing sugars decreased gradually as the rhizome attained maturity (**Figure 5**).There was a steep decline in sugar during the growth phase of rhizome, because it acts as an active source for supply of energy. Reduction of total and reducing sugars was very significant on the 180th day of harvest. The decrease during the maturation phase of rhizome may be attributed to formation of storage components such as starch and its derivatives. Structure, biochemical components, and functional properties of mango ginger starch is well documented (*3*). The starch is essential to protect the energy resource of rhizome during the dormant stage. It may acts as a store house of sugars that are necessary for the development of shoot, as in other tubers (*32*).

The protein concentration increased gradually during the growth and maturation period. The concentration ranged from 6.5 to 11.1 mg/100 g, with a peak accumulation period at 180 days (**Figure 6A**). Increase in concentration of protein is in accordance with increase in phenolics and difurocumenonol. The

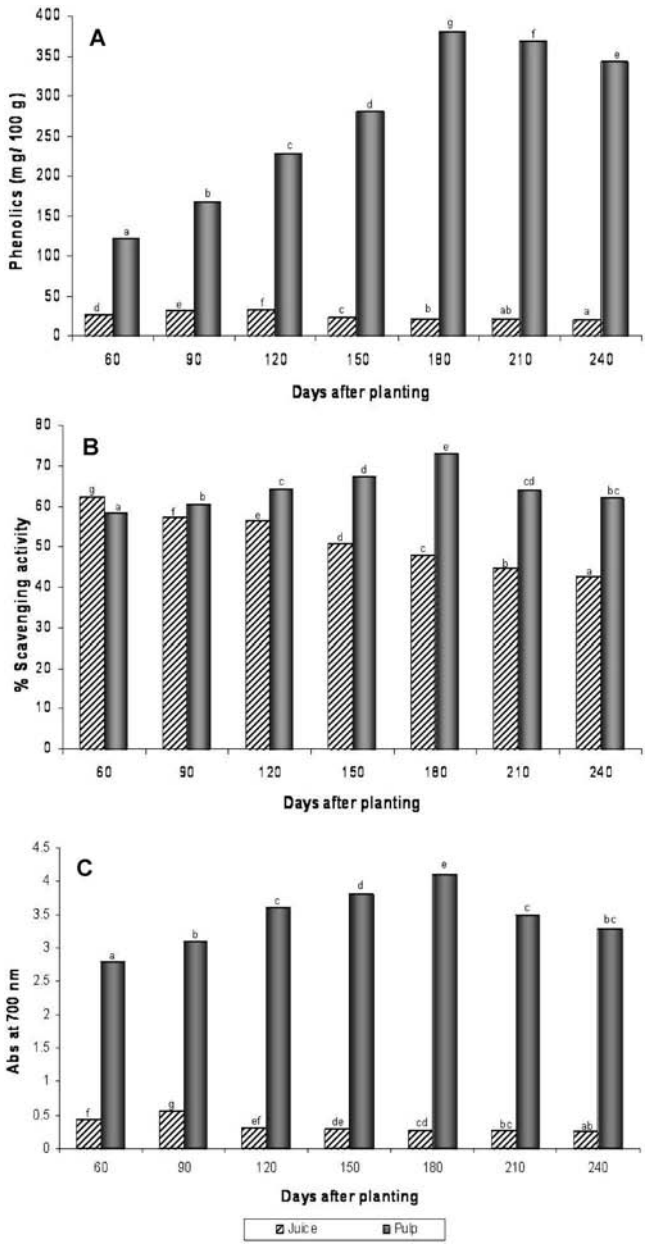

Figure 4. Changes in phenolic content (**A**), DPPH radical scavenging activity (**B**), and total reducing power (**C**) of mango ginger rhizomes during developmental stages. Each value is a mean of three different observations. Values showed by different letters for each line are significantly different at $p < 0.05$.

accumulations of storage proteins were reported in tubers like potato, sweet potato, yam, taro, and cassava, where the major role was to act as stores of nitrogen, sulfur, and carbon, which are required to survive periods of adverse conditions and to provide nutrients for shoot formation (*33*). The storage proteins also exhibit biological activities that are consistent with a role in protecting the tubers against pests, pathogens, and also abiotic stresses as antioxidants and enzyme inhibitors (*33*). Protein abundance decreased during the senescence phase (**Figure 6A**). Protein synthesis and accumulation is a complex phenomenon governed by the physiological and abiotic factors during developmental stages of the rhizome.

pH, Titrable Acidity, and Total Soluble Solids. The pH of mango ginger juice increased gradually with an advance in maturity and was highest on the 120th day. Later it remained constant throughout with a pH range of 6.2–6.4 (**Figure 6B**).

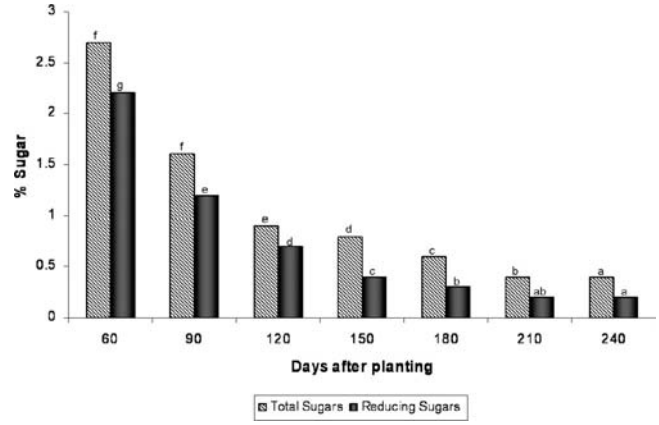

Figure 5. Changes in total sugar and reducing sugar content of mango ginger rhizomes during developmental stages. Each value is a mean of three different observations. Values showed by different letters for each line are significantly different at $p < 0.05$.

A gradual increase in percentage of total solubles with increase in growth and advancement of maturity of mango ginger rhizome was noticed until 120 days. The trend was reversed, recording a decrease up to 240 days, because the solubles, which mainly contains sugars, form a source of energy for various physiological functions that varied with different developmental stages of mango ginger rhizome. In contrast, titrable acidity of mango ginger gradually increased throughout the growth and developmental phases of mango ginger rhizome (**Figure 6**C).The increased or decreased concentration of pH and titrable acidity along with TSS appears to be governed by the variation in composition of cellular metabolites and their functions. It is interesting to note that the point of intersection between total soluble solids and acidity coincides with 150 days (**Figure 6C**) of developmental stages of mango ginger. This point of may terminate the growth phase and initiate the onset of maturation.

Maturity Markers for Mango Ginger. The present study clearly indicated that the synthesis and accumulation pattern of difurocumenonol, phenolics, and protein concentrations served as bioactive markers to determine the physiological maturity for harvest of the mango ginger rhizomes. Difurocumenonol was first observed in 120 day old rhizomes after planting, while phenolics and protein accumulation were detected 60 days after planting. However, they follow a similar pattern of accumulation as a function of growth and maturation of the rhizome. During the growth phase there was a gradual increase in concentration of phenolics, difurocumenonol, and total proteins, while their accumulation was maximum after 180 days. High concentration of soluble and storage components along with bioactive compounds is of paramount important, because their concentrations depleted with delay in harvest after 180 days, which indicates the onset of senescence phase. The distinct patterns of these biochemical markers were associated with conspicuous display of drying and detachment of leaves from the rhizome. This provides a visual clue for maturation of rhizome in mango ginger plant. The various maturity indices displayed on 180 days (**Table 1**) from planting were found to be optimum for harvest, compared with the conventional harvest ranges from 200 to 240 days.

For the first time a new set of optimum maturity indices for harvest of mango ginger rhizome have been established, based on the synthesis and accumulation pattern of the bioactive terpenoid difurocumenonol and phenolics. Interestingly, they

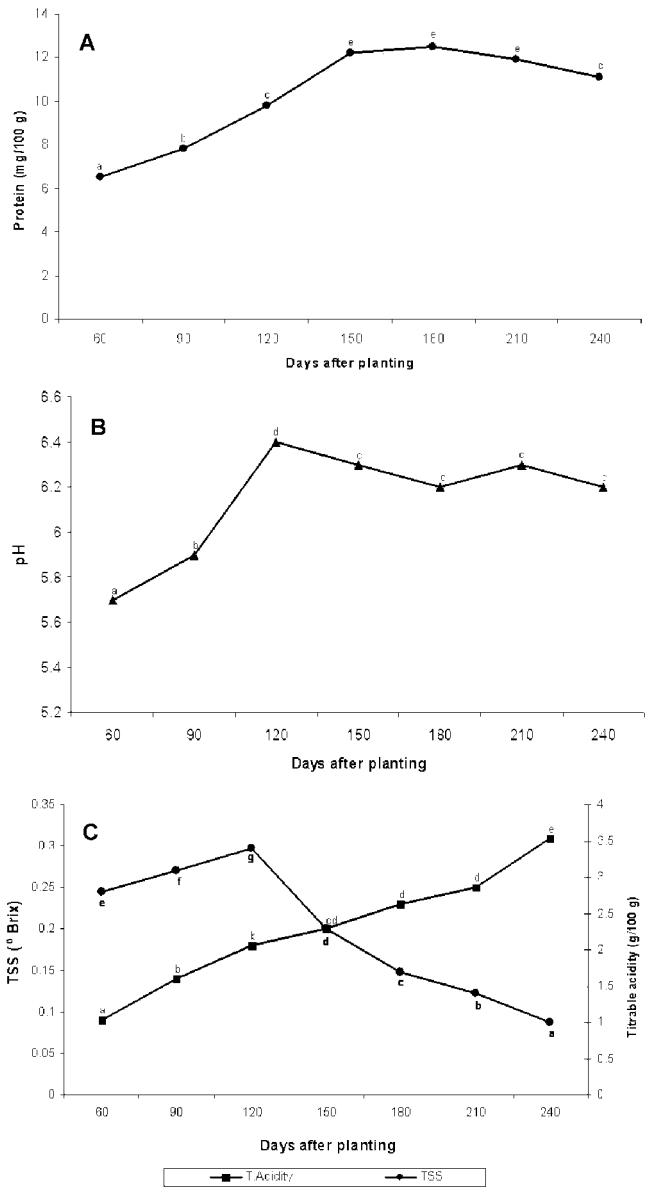

Figure 6. Changes in total protein content (**A**), pH (**B**), TSS, and titrable acidity (**C**) mango ginger rhizomes during developmental stages. Each value is a mean of three different observations. Values shown by different letters for each line are significantly different at $p < 0.05$.

Table 1. Maturity Standards for Harvest of Mango Ginger Rhizome

maturity markers	index
Biochemical	
phenolic content (mg/ 100 g)	380
difurocumenonol concentration (mg/100 g)	34
protein content (mg/100 g)	12
Morphological	
drying and detachment of leaves (days)	180
Rhizome Characteristics	
size of rhizome: (a) length (cm)	12–13
size of rhizome: (b) diameter (cm)	3–4
lemon yellow pigmentation of pith region (days)	180
pith and cortex ratio of rhizome	2:1

demonstrated a distinct pattern of accumulation governed by the physiological maturity of rhizome and age of the mango ginger. Development of mango ginger rhizome and its maturation are directly influenced by coordinated alterations of several biochemical factors. The importance of these patterns remains to be determined.

ACKNOWLEDGMENT

We are thankful to Dr. V. Prakash, Director, CFTRI, Mysore, India, for his keen interest in the work and encouragement. We also thank R. Ravi, Scientist, Department of Sensory Science, CFTRI, Mysore, for his help in statistical analysis.

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Received for review May 27, 2007. Revised manuscript received July 24, 2007. Accepted July 24, 2007. R.S.P. thanks the CSIR, New Delhi, India, for awarding the Senior Research Fellowship.

JF0715469