Chapter 4

Functional Properties and Biochemical Changes in Mango Ginger Rhizomes during Storage

If I have seen further than others, It is by standing upon the shoulders of giants.  
-Isaac Newton
Introduction

Underground storage organ vegetables generally are subject to a number of physiological disorders that limits the ability of postharvest technology to maintain freshness and quality. Root vegetables are also susceptible to high water loss, a major limitation for postharvest storage [Burton, 1982], and various bacteria and fungi that are present in the soil cause decay during storage [Brecht, 2003]. Postharvest losses of vegetables have been estimated to be as high as 25-50 % due to poor postharvest handling and storage temperature management [Nunes and Emond, 2003]. Postharvest deterioration of mango ginger includes physiological loss of water, and shriveling followed by sprouting. The antioxidant components of rhizome are highly susceptible to postharvest physiological stress, temperature and duration of storage. Tropical and subtropical root vegetables are also susceptible to chilling injury below 7°C, which is accompanied by a loss of flavour and sprouting [Ravi and Aked, 1996].

Temperature is the most critical factor that alleviates or aggravates the physiological and bioactivity in mango ginger rhizome after harvest. In areas in which cold storage facilities are not available, it is a common practice to rebury the rhizomes or leave them unharvested. Because of high medicinal properties and its importance in the food industry as a source of raw mango flavour and for its nutraceutical properties, there is a need to retain quality after harvest. Hence, storage studies of freshly harvested rhizomes were planned to evaluate the antioxidant activity and biochemical quality changes as a function of storage temperature and time. This investigation also aimed to find out optimum storage temperature to extend the shelf life of mango ginger rhizome. The details of the work carried out presented in this chapter.
**Materials and Methods**

**Storage conditions and sample size**

Mango ginger rhizomes were harvested fresh from the commercial farm at Sultan’s Bathery, Kerala, India. They were immediately transferred to laboratory. The rhizomes were washed, air-dried and stored in plastic mesh basket at three different temperatures; [1] Room Temperature [RT] [25 ± 1°C]; [2] Low temperature [LT] [14 ± 1°C]; [3] Chilling temperature [CT] [4 ± 1°C], with three replicates of 8 kg each. The samples were analyzed periodically at different time intervals: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 days. All analysis was carried out in triplicates.

**Weight loss, chilling injury and sprouting**

Weight loss was measured at different time intervals using an electronic scale. Weight loss during postharvest storage was determined by subtracting sample weights from their previous recorded weights and presented as % of weight loss compared to initial weight. The rhizomes stored at CT were subjected to analysis of susceptibility to chilling injury which is characterized by surface lesions, discoloration, water-soaked tissue, internal browning, loss of mango flavor, and softening of tissue. Similarly, the rhizomes stored at RT were observed for initiation and elongation of sprouts throughout the storage period.

**Biochemical composition**

**Sample preparation**

About 500 g of rhizomes stored at RT, LT and CT were sliced and 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, TSS, sugar content, protein content, phenolic content and antioxidant activity.

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 till it became colourless. The methanolic extract was filtered, and evaporated using a rotary evaporator [Buchi Rotavapor R-124, Switzerland]. The extract was dissolved and diluted to a final volume of 100 ml with 80 % methanol. The mixture was centrifuged at 8000 rpm at refrigerated
temperature [4°C] for 20 min and used for determination of total phenolic content and DPPH radical scavenging activity.

**Determination of phenolics**

The total phenolic content in mango ginger juice as well as pulp was determined using a modification of 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’s 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 a standard curve of gallic acid.

**DPPH radical scavenging activity**

DPPH [1, 1-diphenyl-2-picrylhydrazyl] radical scavenging activity was determined according to a method previously described [Blois, 1958; Bondet et al., 1997]. 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 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.

**pH, titrable acidity and total soluble solids**

pH of the fresh juice was measured using a Control Dynamics pH meter calibrated with standard buffer pH 7. Titrable acidity was determined by the AOAC [1990] method. The total soluble solids [TSS] were determined by a digital refractometer [ATAGO RX-5000, ATAGO, Japan] calibrated with distilled water. Mango ginger juice was passed through a filter paper Whatman No.1 using vacuum before analysis.

**Reducing sugars, total sugars and total protein contents**

Reducing sugars and total sugars were determined by using the method as described by Ranganna [2001]. The total protein content was determined by the Bradford method [1976], using bovine serum albumin [BSA; Sigma Chemical, St. Louis, USA] as a standard protein.
**Extraction and quantification of difurocumenol by HPLC**

To study the concentration of difurocumenol, the fresh rhizomes [10 g] were homogenized with chloroform till they became colorless. The extract was filtered and concentrated using a rotary evaporator and freeze dried before using the sample for HPLC analysis. Difurocumenol [the isolated compound] and chloroform extracts obtained from mango ginger rhizomes stored at different temperatures at different storage time were tested using a LC-10AT liquid chromatograph [Shimadzu, Singapore] equipped with 300 x 4.6 mm i.d., 5 μ, Thermo Hypersil C-18 column [Bellefonte, PA, USA]. The gradient programme used for 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; 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.
Results and Discussion

Harvest causes an artificial interruption of the rhizome’s natural life cycle, where water can no longer be taken up from the soil, while biochemical changes and antioxidant activity are difficult to interrupt. The physiological changes and antioxidant activity of rhizomes at three different temperatures, Room temperature [RT], low temperature [LT] and chilling temperature [CT] were studied. The rhizomes stored at RT and LT were analyzed periodically for biochemical changes and antioxidant activity up to 120 days, and CT stored rhizomes only for 60 days because of spoilage due to chilling injury.

Water loss, sprouting and chilling injury

The cut portion of the mango ginger rhizome forms the main avenue for increased loss of water due to increased rate of respiration and transpiration that are governed directly by storage temperature and time. Water loss was higher in the rhizomes stored at room temperature than at low temperature [Fig. 4.1].

Rapid loss of water at a rate of 1 % per day occurred in rhizomes during the initial 30 days, and later at a rate of 0.07 % until 120 days of storage at RT. About 33 to 36 % of water loss on the 50th day of storage from the time of harvest coincides with manifestation of visible shriveling of the rhizomes. Further water loss of 3 to 4 % leads to a commercially objectionable level of shriveling and rhizomes become unsalable. Many vegetables
become unsalable and manifest shriveling after loosing 7-20 % of their weight [Ben-Yehoshua and Rodov, 2003]. Rhizomes stored at 14°C and 4°C lost about 8 and 2 g/kg/month respectively. The pivotal physiological role of water in mango ginger is maintenance of rhizome turgidity, freshness and quality as is the case for most perishables [Herppich et al., 2000].

The initiation of sprouting was observed on the 80th day in rhizomes stored at RT [Fig. 4.2]. There was a significant increase in protein content prior to sprouting of rhizome at RT, which may be due to de novo synthesis of proteins. Increase in proteins and nucleic acids prior to sprouting were observed in many vegetables [Macdonald and Osborne, 1988]. Protein synthesis required for sprouting, while nucleic acid synthesis for sprout elongation [Madison and Rappaport, 1968; Alam et al., 1994].

Rhizomes stored at CT failed to sprout due to chilling injury. The potential symptoms of chilling injury were water-soaked lesions with tissue softening and browning [Fig. 4.3]. The injured tissue eventually lost its characteristic mango flavour followed by off-odours. Until recently, it was believed that chilling injury involved membrane modification and changes in proteins [Hausman et al., 2000] and increased permeability and ion leakage [Murata, 1990; Saltveit, 2002]. Extensive studies have demonstrated that ion leakage occurs from chilling injured, yet living cells [Palta, 1990]. Hence, rhizomes could not be stored for more than 30 days at CT.
Total phenolics

The total phenolic content in fresh juice was 20.8 mg/100 g. It showed an increasing trend both in RT and LT stored rhizomes. Low temperature favored significant accumulation of total phenolics throughout the storage period. The chilling temperature induced the highest concentration of 58.8 mg/100 g of phenolics within a short duration of 30 days, but this declined sharply to 10 mg/100 g on the 60th day [Fig. 4.4A].

The phenolic content of rhizome pulp was 20 times higher than that of the juice. It remained unchanged until the 60th day but decreased with extended time of storage at RT. Low temperature favored the accumulation of phenolics and it increased from 380 to 568 mg/100 g for a storage period of 120 days. Chilling temperature resulted in an increase in phenolics in rhizome pulp for a period of 30 days [Fig. 4.4B]. The accumulation of phenolics in rhizomes stored at CT could inhibit or slow down sprouting because of their affinity towards oxygen [Cvirkova, et al., 1994]. Several secondary metabolites, including phenolics accumulate to mediate the chilling temperature and other stresses [Christie et al., 1994]. Subsequent browning of chilling injured rhizome tissues may be due to the interaction between phenols and polyphenol oxidase which are generally found in separate compartments in the cell [Crisosto et al., 1999].
**Antioxidant activity**

Room temperature [RT] storage negatively affected antioxidant activity, both in mango ginger juice and pulp, showing a downward trend [Fig. 4.5A and 4.5B] throughout the storage time. The antioxidant activity of the juice remained unchanged or increased slightly after 80 days at LT. Increasing trend in accumulation of phenolics was exhibited both in juice and methanolic extract of pulp stored at LT. Accumulation of phenolics has been shown to be in response to a range of biotic and abiotic stresses [Dixon and Paiva, 1995; Solecka and Kacperska, 2003].

Chilling temperature affected antioxidant activity positively both for juice and pulp for a period of 30 days followed by a decline with the advance of time. The antioxidant activity of many fruits and vegetables are mainly attributed to phenolic compounds [Kalt, et al., 1999]. However, antioxidant activity of mango ginger appears to be independent of total phenolics.
**pH, titrable acidity and total soluble solids**

The fresh mango ginger juice had a pH of 6.6 immediately after harvest. It remained stable in rhizomes stored at RT and LT. However, storage at CT resulted in an increase from 6.3 to 6.9 from 40 to 60 days [Fig. 4.6A]. Titrable acidity measured as citric acid showed a downward trend in all the three storage temperatures [Fig. 4.6B]. The decrease in acidity was highly significant at CT when compared to RT and LT. Loss of acidity was least in LT [0.11 %] when compared to RT [0.05 %].

A steady increase of 1% in total soluble solids was exhibited in the mango ginger on the 120th day at RT. Low temperature exerted a dual effect on TSS, with an initial decrease of 1.5 % until 30 days followed by a gradual increase [2.7 %] to 120 days. There was no significant change in the TSS of rhizomes stored at CT [Fig. 4.6C].
Total sugars and reducing sugars

Freshly harvested mango ginger had 0.95 % and 0.42 % total sugars and reducing sugars respectively. Rhizomes showed a decrease in total sugars and reducing sugars by 0.53 and 0.10 % respectively up to 60 days of storage at RT. An interesting observation was an increase in total sugars and reducing sugars from the 80th day onwards during storage. A sudden increase in both the sugars by 0.82 and 0.56 % respectively was observed up to 120 days [Fig. 4.7A and 4.7B]. Shift in nutritional and metabolic sink of rhizomes, due to harvest causes severe physiological stress. This was effectively overcome initially at the expense of total sugars and reducing sugars, acidity and TSS. Thus the increase in sugars which may be attributed to hydrolysis of starch into simple sugars [Biale, 1960]. CT influenced accumulation of both sugars after 30 days of storage. With an increase in time, there was a linear increase in concentration of total sugars and reducing sugars throughout the storage period. Low temperature sweetening by accumulation of total and reducing sugars was exhibited by rhizome. The process of sweetening is accompanied with conversion of starch to sugars. This phenomenon could also be explained by the glucose consumption by the respiration, which was inhibited at the LT [Salunkhe and Desai, 1984; Brecht, 2003]. High temperature negatively influenced the rhizome by attenuating the metabolic activity resulting in decrease in total sugars and reducing sugars.
Total protein content

Total protein content of freshly harvested mango ginger rhizome was 11.2 mg/100 g, which was reduced to 7.9 mg on the 70th day in RT storage. Further, storage of rhizomes at RT showed an increase in protein content up to 10.6 mg/100g on the 120th day [Fig. 4.8]. Low temperature also exerted a similar trend on protein content of rhizomes, i.e. an initial sudden decline in the protein content to 8.6 mg/100 g for 10 days of storage followed by slight increase [11.3 mg] on the 120th day. Chilling temperature negatively affected total protein content of the rhizomes and this reached the lowest concentration of 3.2 mg/100 g on the 60th day. There was a significant increase in protein content prior to sprouting of rhizome at RT, which may be due to de novo synthesis of proteins. Increase in proteins and nucleic acids prior to sprouting were observed in many vegetables [Macdonald and Osborne, 1988]. Protein synthesis required for sprouting [Madison and Rappaport, 1968; Alam et al., 1994].

Effect of storage temperatures on difurocumemononol

The concentration of difurocumemononol in mango ginger rhizomes during storage up to 120 days was carried out using HPLC. Difurocumemononol [Fig. 4.9] - a terpenoid compound, has been characterized and reported to have antimicrobial activity against a wide range of microbes [Policegoudra et al., 2007]. This has been used as marker to evaluate the quality of mango ginger rhizomes during storage at different temperatures. The highest concentration of difurocumemononol [40 mg/100 g] was noticed in 70 days old rhizomes stored at RT and decreases thereafter till 120 days [Fig. 4.9A]. This may be due to the loss of water rather than de novo synthesis of compound. The peak accumulation of difurocumemononol at RT storage associated with conspicuous display of shriveling, a visual
quality marker of rhizome. Later decrease in its concentration heralds the onset of sprouting a major limiting factor to extend the shelf life of rhizome at RT. At CT difurocumenonol exhibited slight increase in concentration till 30\textsuperscript{th} day followed by a drastic decrease [\textbf{Fig. 4.9C}]. Changes in difurocumenonol during storage at CT appear to be influenced by chilling injury. At LT gradual decrease in the concentration of difurocumenonol was observed with increase in storage period [\textbf{Fig. 4.9B}]. It is interesting to note that difurocumenonol exhibited different pattern of accumulation as a function of temperature during storage period.

\textbf{Fig. 4.9: Effect of different temperatures on difurocumenonol during storage of mango ginger rhizomes}

\begin{itemize}
  \item \textbf{A}: Room temperature
  \item \textbf{B}: Low temperature
  \item \textbf{C}: Chilling temperature
\end{itemize}
Effect of temperatures on shelf-life

Room temperature aggravates rapid depletion of water, increased TSS, sugars, phenolics and antioxidant activity in mango ginger rhizome. These changes may be responsible for short shelf life of 3 months at RT, beyond which shriveling and sprouting makes rhizomes unsalable. Chilling temperature negatively affected the rhizome and showed a ‘bell’ shaped curve, which is characterized by, initial accumulation of TSS, total sugars, phenolics that decrease rapidly in later stages. The peak period of accumulation of the above chemical constituents on 30th day may heralds the onset of chilling injury and thus limits the storage period. Low temperature [14±1°C] was found to be optimum for storage of mango ginger rhizome. It alleviates excessive moisture loss, avoids chilling injury, delay sprouting, and ultimately extends the shelf life of rhizome by 4-5 months. Thus, biochemical changes, antioxidant activity and shelf-life of mango ginger are directly governed by temperature and time of storage.

Conclusion

This is the first report on biochemical changes and antioxidant activity of mango ginger at different storage temperatures. Within the range of temperatures, lower end [4°C] was characterized by chilling injury manifested by discoloration, softening, browning of internal tissue, loss of quality and failure to sprout, whereas, upper end [24°C] was characterized by sprouting, shriveling. Moderate low temperature [14°C] is optimum for storage of mango ginger. It retained mango flavour, and minimized the changes in composition, and also biochemical and antioxidant properties. It reduced excessive moisture loss, avoid chilling injury, delay sprouting, maintain freshness, ultimately extended the shelf life for 4-5 months. This suggests that mango ginger rhizome quality is a function of temperature and time during storage and exerts a decisive role in determining its biochemical quality. For the first time difurocumenonol a multi functional bioactive compound has been successfully used as biomarker to asses the quality changes in mango ginger rhizome during storage. These aspects, which I believe have commercial applications to obtain rhizome with high bioactive components a preferred quality for preparation of pharmaceutical of nutraceutical products.