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CHANGES IN VITAMIN C CONTENT DURING THE VARIOUS STAGES OF RIPENING OF *CITRUS GRANDIS* - A MAJOR FRUIT CROP OF SIKKIM, INDIA

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ABSTRACT
Ascorbic acid is the most important antioxidant in fruit juices. It protects the organism from oxidative stress thereby reducing risk of developing some chronic diseases. The present project work was carried out to determine the antioxidant capacity (measured in terms of ascorbic acid content) during the various stages of ripening of *Citrus grandis*, a major fruit crop of Sikkim. The fruits of ten different plants of *Citrus grandis* were plucked at different stages and were analyzed for total acidity and vitamin C content. The total antioxidant capacity in terms of ascorbic acid and total acidity were determined using standard dye titration method using 2,6-dichloroindophenols dye (DCIP) and titration method against sodium hydroxide using phenolphthalein indicator respectively. Acidity in terms of citric acid content was found to be more in the unripe juice (3.9g/100mL) and less in ripe juice (2.7g/100mL). It was observed that unripe fruits of *Citrus grandis* have higher antioxidant activity (52 mg/100mL) in terms of ascorbic acid content than the ripe fruits (36 mg/100mL). For nutritional and medicinal uses, the fruits of *Citrus grandis* can be consumed for its appreciably high ascorbic acid contents.

INTRODUCTION

Due to its nutritional value and flavor, *Citrus* is one of the fruits being consumed fresh or as juice. These fruits contain a variety of sugars, citric acid, ascorbic acid, carotenoids, minerals, essential oils, *etc* and play an important role in human nutrition as an excellent source of antioxidants (Rekha *et al*., 2012). Ascorbic acid is the most important vitamin and natural antioxidant in fruit juices especially in *Citrus sp*. More than 90% of the vitamin C in human diets is supplied by fruits and vegetables (Khomdram and Devi, 2010). Vitamin C is defined as the generic term for all compounds exhibiting the biological activity of L-ascorbic acid (Stryer, 1988).

Ascorbic acid is required for the prevention of scurvy and maintenance of healthy skin, gums and blood vessels. It reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer (Sarkar *et al*., 2009; Swapana *et al*., 2012, Pal *et al*., 2013). Ascorbic acid also protects the organism from oxidative stress (Ebrahimzadeh *et al*., 2004; Fernandez *et al*., 2005) and is also shown to possess anti-inflammatory, antioxidant, antitumor and antifungal activities (Ghafar *et al*., 2010). Because of these health benefits, the consumption of fruit juices is beneficial and the health effects of fruits are ascribed, in part to ascorbic acid, a natural antioxidant (Rekha *et al*., 2012). Due to favorable climatic condition, *Citrus grandis* (pomelo) is one of the major fruit crop in Sikkim. The fruit is usually pale green to yellow when ripe, with sweet white (or, more rarely, pink or red) flesh and very thick albedo (rind pith). Considering the importance of fruits in human health which is used for prevention of various diseases, the present work is conducted to study the content of ascorbic acid and its changes during the different stages of ripening of *Citrus grandis*.

MATERIALS AND METHOD

Extraction of juice

Ten different plants (Fig.1) of *Citrus grandis* from different localities in and around Marchak, Gangtok, Sikkim were selected for the study. The fruits from the plants were plucked at different stages of ripening. The juices were extracted from fruits developed after 12-14 weeks of flowering. After every two weeks the juices were analyzed for pH, acidity and ascorbic acid content. The fruits were washed thoroughly in water. The peel was removed carefully avoiding any cut or damage to the pulp. The juice was extracted by mechanical squeezing by cutting the fruits in half. The collected juice was filtered through muslin cloth, centrifuged at 6000 rpm for 5mins. Supernatant was collected and used for the present study. All the materials used in the preparation of the juice were first thoroughly washed by distilled water and dried in hot air oven in order to avoid dilution of the fruit juices and other unwanted chemical reaction. All the results were expressed as mean of triplicate determination from each of the ten plants.

Determination of pH and total acidity

pH of the juices of different stages of fruit were determined by using Eutech pH meter (EC pH 1500 - 42s) after standardization with pH 4, pH 7 and pH 10**.** The total acidity of the fruit juice was determined by standard protocol of AOAC (2000) by titrating the samples using phenopthalein indicator with 0.1N NaOH which was standardized using standard oxalic acid. The end point was noted when the colour changed from colorless to pale pink. Total acidity was calculated in terms of citric acid using formula, Acidity (g/100 mL) = Normality of the juice x Equivalent weight of citric acid.

Estimation of Ascorbic acid content

The ascorbic acid content of the juices from different stages ripening was determined using standard protocol of AOAC, 2000 using dye-titration with 2,6-dichloroindophenols dye (DCIP). 5 mL of standard ascorbic acid was taken in a conical flask containing 10 mL 4% oxalic acid and was titrated against the 2,6-dichlorophenol indophenols dye. The appearance and persistence of pink colour was taken as end point. The amount of dye consumed (V_1 mL) is equivalent to the amount of ascorbic acid. 5 mL of sample was taken in a conical flask having 10 mL of 4% oxalic acid and titrated against the dye (V_2 mL). The amount of ascorbic acid was calculated using the formula, Ascorbic acid (mg/100 g) = (0.5mg/V_1mL) × (V_2/15 mL) × (100 mL/Wt. of sample) × 100.

RESULTS AND DISCUSSION

Citrus grandis (the biggest citrus in the world) has a sweet and deliciously tart taste. Fruits for marketing are generally harvested when they begin to turn color. The dull skin of the unripe fruit brightens upon ripening as the oil glands in the rind becomes more prominent and shiny (Ibrahim, 2000). With regard to the climatic condition in Sikkim (Marchak), India, the fruits are observed to mature about 5.5-6 months after flowering. For the purpose of our study, analysis of the fruits was performed after 3 months of flowering. The fruits were plucked after every 2 weeks starting from the 3rd month after flowering. The fruits were analyzed for its pH, total acidity and ascorbic acid content.

An increase in the pH was observed from unripe to ripe fruit during the various stages of ripening process (2.06 to 3.12). The pH was found to be lesser in unripe fruits when compared to ripe fruits which indicate that unripe fruit is more acidic than the ripe fruit. The total acidity was found to higher in unripe fruit (3.992g/100mL) when compared to ripe fruits (2.766g/100mL) (Fig.3). Acidity in terms of citric acid content was found to be more in the unripe juice and less in ripe juice. The ascorbic acid content which was estimated titrimetrically was found to be highest in unripe fruits i.e. at 12 weeks after flowering which was 52.0 mg/100mL. It was observed that there was a gradual decrease in the ascorbic acid content during the subsequent ripening process and when the fruit is fully ripped and suitable for consumption, the ascorbic acid decreases to 36.0mg/100mL which was after 26 weeks after flowering (Fig.4). It was observed that unripe fruits of *Citrus grandis* have higher antioxidant activity in terms of increased ascorbic acid content (52 mg/100mL) than the ripe fruits (36 mg/100mL). Kumari *et al.*, 2013 has also observed that there is decrease in the ascorbic acid content of ripe fruits as compared to unripe fruits of *Citrus aurantifolia* (0.178mg/mL to 0.127mg/mL). Similar findings of decrease in ascorbic acid content during ripening process were recorded by Rekha *et al.*, 2012 for other *Citrus* fruits like *Citrus limon* (10.60 g/100mL from 12.70 g/100mL), *C. reticulata* (6.34g/100mL from 7.41g/100mL), *C. sinensis* (17.40 from 19.04), *C. aurantium* (24.90 g/100mL from 26.01 g/100mL). Moreover, it was observed by Gardner *et al.*, 2000; Zvaigzne *et al.*, 2009 that antioxidant activity was found to be higher in fruit juices containing high ascorbic acid.

Decrease in the ascorbic acid content during ripening process can be attributed to the changes that occur during the process of fruit ripening. Unripe fruits have high amount of ascorbic acid, phenolic, starch, chlorophyll, pectin, acids and organics. During ripening, ethylene (a phytohormone) is released which activates the transcription genes for the synthesis of various enzymes which degrade the phyto-constituents involve in

Figure 1: Citrus grandis

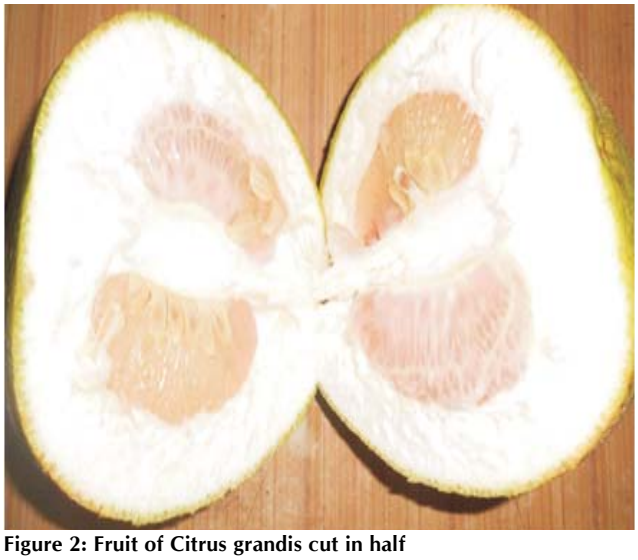

Figure 2: Fruit of Citrus grandis cut in half

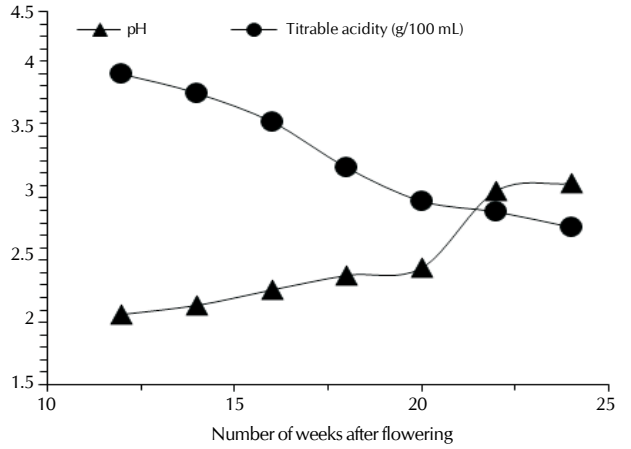

Figure 3: Changes in pH and titrable acidity during ripening of *Citrus grandis* fruit

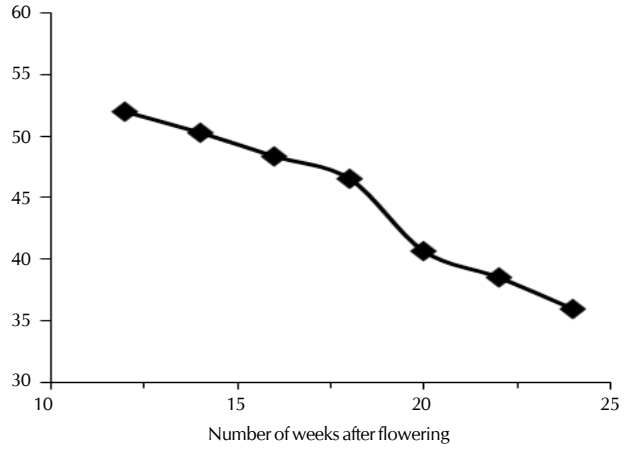

Figure 4: Changes in ascorbic acid content during ripening of *Citrus grandis* fruit

ripening process. During ripening metabolism speeds up and also a number of free radicals are generated. Antioxidants like ascorbic acid, phenolics, etc. come for rescue and detoxify the generated free radicals into harmless reduced substances. Thus, there is a reduction in the ascorbic acid, phenolic and other antioxidant contents in the fruits while ripening as a result of which the antioxidant activity of ripe fruit juices is comparatively less than that of unripe fruit juices (Jacob *et al.*, 1999).

In recent years, there is an increasing interest in finding antioxidants from natural origin (Chung *et al.*, 2006). The present results have shown that *Citrus grandis* which are consumed for their nutritional value also have antioxidant activity in terms of appreciable ascorbic acid content. Even though the presence of ascorbic acid is high in unripe fruits of *Citrus grandis* , consumers does not prefer it for consumption because of its tart taste and high acidity. The ripped fruits of *Citrus grandis* also contains appreciably high amount of ascorbic acid and as such can be used as a good source of ascorbic acid and antioxidant. Growing of these fruits will help to reintroduce their use as an alternative source of natural antioxidant. The global consumption of pomelo is steadily increasing but is still low compared to other major citruses. Marketing and consumption of pomelo fruit should be encouraged for nutritional food security.

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